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Seroprevalence and associated risk factors of chikungunya, dengue, and Zika in eight districts in Tanzania

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ABSTRACT

Background: This study was conducted to determine the seroprevalence and risk factors of chikungunya (CHIKV), dengue (DENV), and Zika (ZIKV) viruses in Tanzania.

Methods: The study covered the districts of Buhigwe, Kalambo, Kilindi, Kinondoni, Kondoa, Kyela, Mvomero, and Ukerewe in Tanzania. Blood samples were collected from individuals recruited from households and healthcare facilities. An ELISA was used to screen for immunoglobulin G antibodies against CHIKV, DENV, and ZIKV.

Results: A total of 1818 participants (median age 34 years) were recruited. The overall CHIKV, DENV, and ZIKV seroprevalence rates were 28.0%, 16.1%, and 6.8%, respectively. CHIKV prevalence was highest in Buhigwe (46.8%), DENV in Kinondoni (43.8%), and ZIKV in Ukerewe (10.6%) and Mvomero (10.6%). Increasing age and frequent mosquito bites were significantly associated with CHIKV and DENV seropositivity ($P < 0.05$). Having piped water or the presence of stagnant water around the home ($P < 0.01$) were associated with higher odds of DENV seropositivity. Fever was significantly associated with increased odds of CHIKV seropositivity ($P < 0.001$). Visiting mines had higher odds of ZIKV seropositivity ($P < 0.05$).

Conclusions: These findings indicate that DENV, CHIKV, and ZIKV are circulating in diverse ecological zones of Tanzania. There is a need to strengthen the control of mosquito-borne viral diseases in Tanzania.

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Introduction

Globally, arthropod-borne viral diseases account for 17% of all human infectious diseases (Jones et al., 2008; Kading et al., 2020). Of the arboviral diseases, mosquito-borne diseases are the most important, affecting millions of people, and comprise an important proportion of emerging and re-emerging human pathogens. Dengue, yellow fever, Japanese encephalitis, chikungunya, and Rift Valley fever have been described to contribute highly to disability-adjusted life years (Labeaud et al., 2011; Stanaway et al., 2016; Zeng et al., 2018). Global human population growth, unplanned settlements, international travel, and climate variability/change are among the factors that contribute to the expansion of the spread of arboviral diseases (Gould & Higgs, 2009; Gubler, 2011; Lambrechts et al., 2010; Braack et al., 2018). This increases the risk of mosquito-borne viral infections through cycles involving human–human and human–peri-domestic *Aedes* mosquito transmission (Braack et al., 2018; Gaye et al., 2019).

Periodic outbreaks related to mosquito-borne viral infections are common in sub-Saharan Africa (SSA) and include Rift Valley fever, dengue, chikungunya, Zika, and yellow fever (Braack et al., 2018). Recent dengue virus (DENV) outbreaks have been reported in Tanzania (Vairo et al., 2012; Ward et al., 2017; Chipwaza et al., 2020), Mozambique (Mugabe et al., 2018), Ghana (Amoako et al., 2018), Sudan (Elaagip et al., 2020), Benin, Cote d'Ivoire, and Mauritius (WHO, 2019). Outbreaks of chikungunya virus (CHIKV) have been reported in Kenya (Kariuki Njenga et al., 2008), the Republic of Congo (Vairo et al., 2020), and the Reunion Islands (Vazeille et al., 2007). Zika virus (ZIKV) infections have been reported in Cape Verde (Kindhauser et al., 2016; Lourenço et al., 2018), Guinea Bissau (Gulland, 2016), Mozambique (Gudo et al., 2016), and Angola (Hill et al., 2019). Although the acute stages of arboviral infections most often cause a broad spectrum of clinical manifestations, ranging from asymptomatic to severe undifferentiated fever (Forshey et al., 2010; Labeaud et al., 2011), they are also causes of fever that is often considered to be malaria by clinicians, especially in areas with inadequate laboratory capacities (Crump et al., 2013; Ayorinde et al., 2016).

Despite the evidence that arboviral diseases such as dengue and chikungunya contribute substantially to morbidity in Tanzania, there are only a few isolated studies that have documented their burden, drivers, and vulnerability (Vairo et al., 2012; Kinimi et al., 2018; Budodo et al., 2020). Most of these studies have been facility-based studies, and little evidence has been based on population-based studies. This means that their distribution in the country remains uncertain. The objective of this study was, therefore, to determine the seroprevalence and risk factors of chikungunya, dengue, and Zika in diverse ecological zones of Tanzania.

Materials and methods

Study sites and design

This cross-sectional study was performed between April and November 2018. A multistage cluster design was utilized to select the study sites. The country was first divided into five distinct ecological zones based on vegetation and land cover, normalized difference vegetation index, rainfall and number of wet days per month, and elevation. Zone 1 comprised the western parts of Tanzania, with tropical forest, a unimodal rainfall pattern, and altitude <2300 m above sea level. Zone 2 included the Southern Highlands districts, with tropical forest, a bimodal rainfall pattern, and elevation >2300 m. Zone 3 comprised the north-eastern part of the country, lying along the Indian Ocean to 1800 m above sea level. Zone 4 covered the central part of the country, characterized by wet savannah with bimodal rainfall (1400 mm) and semi-arid areas characterized by a short wet season (with rainfall of 400–800 mm per year). Zone 5 comprised the Lake Victoria basin, characterized by a bimodal rainfall pattern (900–1800 mm) with a moderate warm climate.

Buhigwe and Kalambo districts were selected to represent the Western zone and Kyela district the Southern Highland zone, while Kilindi and Kinondoni districts represented the North-eastern zone. Mvomero and Kondoa districts were selected to represent the Central zone, while Ukerewe district represented the Lake Victoria zone (Fig. 1). Details of the study sites have been described elsewhere (Rugarabamu et al., 2021).

Sample size and sampling

A recent review on dengue prevalence in Africa reported IgG prevalence of 15.6% (range 9.9–22.2%) for healthy populations and 24.8% (range 13.8–37.8%) for populations presenting with fever (Simo et al., 2019). This study planned to collect data from both the household and health facility settings; thus, based on the ecology of the zones, different levels of expected prevalence (P) were assumed in order to obtain the needed sample sizes. For zones 1, 2, and 4, the average of the healthy and fever populations (P = 20.1%) was used; for zone 3, the average of the upper ranges (P = 30%) was used, since it includes a district (Kinondoni) within a large city. The district in zone 5 is an island and was assumed to have a low prevalence, hence the lower prevalence (P = 9.9%) was used. The design effect of 1.5 was used to account for any clustering effect between the districts within the same zone. With the desired absolute precision of 5% and confidence level of 95%, the minimum estimated sample size was 1792 individuals, with the following breakdown: zone 1: n = 454 (Buhigwe = 255; Kalambo = 198); zone 2: Kyela (258); zone 3: n = 461 (Kilindi = 226; Kinondoni = 235); zone 4: n = 395 (Kondoa = 203; Mvomero = 192); and zone 5: Ukerewe (224). A contingency of 10% was considered to account for non-responses, refusal, and/or missing values. The zonal sample was split using probability proportional to the size of the district population to obtain district-specific sample sizes. The study district population densities per square kilometre for Buhigwe, Kalambo, Kilindi, Kinondoni, Kondoa, Kyela, Mvomero, and Ukerewe were 168.8, 15.98, 37.0, 3300, 46.6, 755.9, 47.05, and 283.02, respectively.

Three wards were selected from each district, and three villages were selected from each of the three wards. A minimum of 14 participants was targeted for each village or health facility. At the village level, four to five households were randomly selected based on the sampling frame obtained from the village office. Once selected, all eligible members of the household were recruited into the study. In the healthcare facility setting, one hospital, two health centres, and four dispensaries were selected in each district. In each facility, the study subjects were recruited on a first-come-first-included basis until the targeted sample size was achieved. Children <9 months old were excluded based on the fact that maternal antibodies acquired through transplacental route and during breastfeeding are likely to lead to false-positive results (Watanaveeradej et al., 2003).

Sociodemographic and clinical data

A semi-structured questionnaire installed on smartphones with a digital data collection tool (AfyaData) (Karimuribo et al., 2017) was used to collect sociodemographic and epidemiological data. The data included age, education level, occupation, history of fever, knowledge about mosquito-borne diseases, household water source, and water storage. All study participants were examined for clinical manifestations suggestive of fever and associated

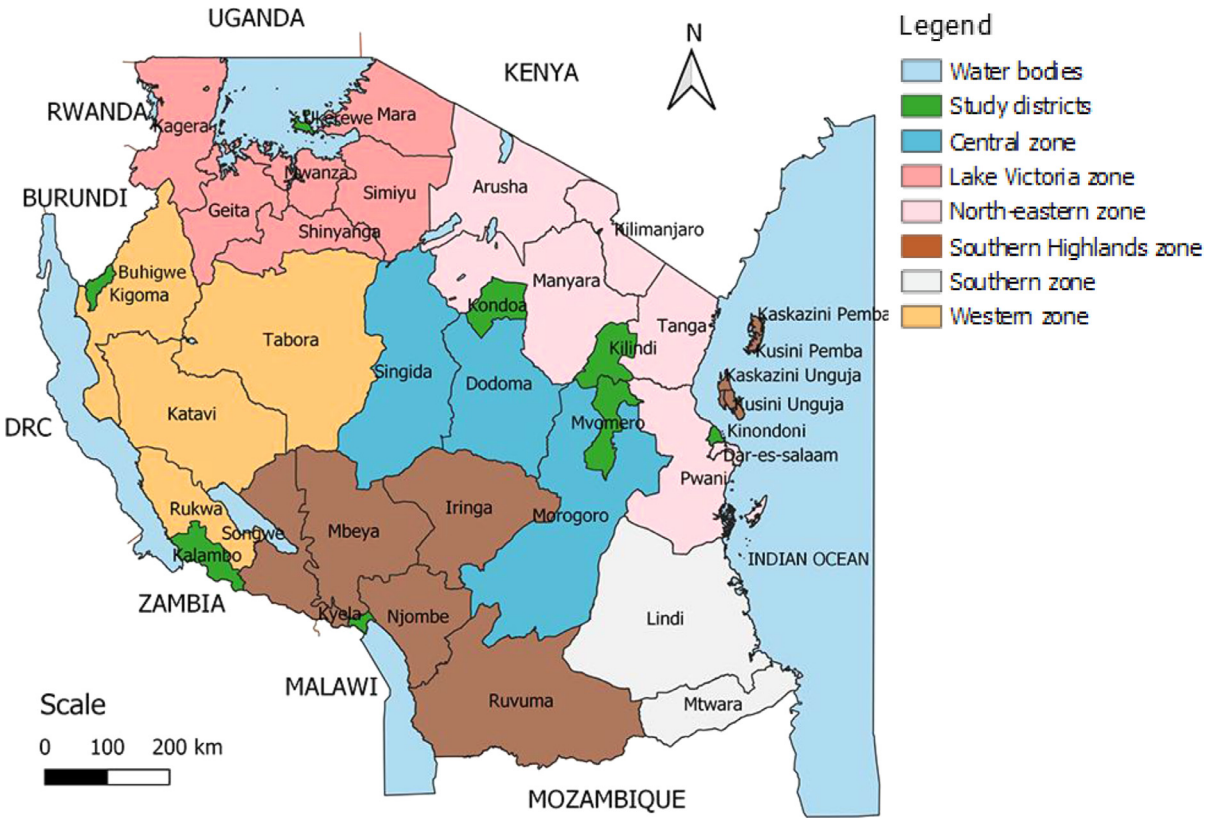

Fig. 1. Map of Tanzania showing the ecological zones and study districts.

disease conditions. Body temperature was recorded using a digital thermometer.

Laboratory analysis

From each participant, about 3–5 ml of blood was collected into a plain vacutainer tube and allowed to clot at room temperature. Thereafter, serum was separated by centrifugation at 3000 rpm for 10 minutes. Serum aliquots of 0.5–1 ml were placed into sterile cryovials labelled with a unique identification code and transported in liquid nitrogen (−196°C) to the laboratory, where they were stored in an ultralow temperature freezer (−80°C) until analysed. Each serum sample was screened in duplicate for human immunoglobulin G (IgG) antibodies against CHIKV, DENV, and ZIKV using EUROIMMUN indirect ELISA test kits (Medizinische Labordiagnostika AG, Lübeck, Germany). The average value of a sample was classified as positive for IgG when the ratio of optical density (OD) of the control or sample over that of the calibrator was ≥1.1, negative when the ratio was <0.8, and borderline when the ratio was ≥0.8 to <1.1. The sensitivity and specificity of the ELISA test kits used were reported to be 98.5% and 95.7% for anti-dengue IgG, 96.8% and 98.0% for anti-chikungunya IgG, and 99.2% and 98.0% for anti-Zika IgG, respectively.

Statistical analysis

Data were imported into Microsoft Excel 2016 (Microsoft Corp, Redmond, WA, USA), cleaned, and organized for analysis. Data were analysed using Stata version 13 (Stata Corp., College Station, TX, USA). Descriptive statistics and frequency tables were used to summarize the data. The Chi-square test was used to determine the association between categorical variables and seroprevalence of CHIKV, DENV, and ZIKV or multiple infections. The variables associated with seroprevalence were subjected to logistic regression analysis to identify significant risk factors for seropositivity. The magnitude of association was measured using the odds ratio (OR) and 95% confidence interval (CI). A variable with a probability value (*P*-value) less than 0.05 was considered statistically significant.

Results

Seroprevalence of DENV, CHIKV, and ZIKV

A total of 1818 participants were involved in the study. They were recruited from 24 wards, 72 villages, and 56 healthcare facilities. Of the facilities involved, eight were hospitals, 16 were health centres, and 32 were dispensaries. The median age was 34 years, with an interquartile range (IQR) of 23–47 years. The overall seroprevalence rates of CHIKV, DENV, and ZIKV were 28.0%, 16.1%, and 6.8%, respectively. The highest seroprevalence rates for CHIKV (43.4%) and ZIKV (10.6%) IgG antibodies were in the Lake Victoria zone. The highest seroprevalence of DENV IgG antibodies was in the North-eastern zone (28.6%) (Table 1). The mean age of CHIKV, DENV, and ZIKV positive individuals was 40, 39, and 38 years, respectively. District-wise, the highest seroprevalence of CHIKV was in Buhigwe (46.8%), of DENV was in Kinondoni (43.8%), and of ZIKV was in Ukerewe (10.6%) (Fig. 2).

Having a secondary education ($P = 0.02$), water bodies around the home, and piped water at home were significantly associated with DENV seropositivity. Fever was significantly associated with CHIKV ($P < 0.001$) and ZIKV ($P = 0.02$) seropositivity. Having visited mines was significantly associated with ZIKV seropositivity ($P < 0.04$). Increasing participant age and experience of frequent mosquito bites were significantly associated with DENV and CHIKV

Table 1
Seroprevalence of immunoglobulin G antibodies (IgG) specific to dengue, chikungunya, and Zika viruses by sociodemographic characteristics, ecological zones, and exposure risk factors

Variable	Category	Number tested	Dengue Number IgG-positive (%)	Chikungunya Number IgG-positive (%)	Zika Number IgG-positive (%)
Sex	Female	989	151 (15.3)	273 (27.7)	59 (5.9)
	Male	829	141 (17.1)	236 (28.6)	62 (7.5)
Age (years)	<28	642	71 (11.1)	128 (19.9)	39 (6.1)
	28–42	607	109 (17.9)	179 (29.5)	42 (6.9)
	>42	569	112 (19.7)	202 (35.5)	43 (7.6)
Education	Primary	1135	183 (16.1)	327 (28.8)	73 (6.4)
	Secondary	219	49 (22.4)	53 (31.9)	16 (7.3)
	Post-secondary	53	5 (9.4)	10 (18.9)	6 (11.3)
	None	411	55 (13.4)	119 (28.9)	29 (7.1)
Occupation	Farming	1067	148 (13.9)	343 (32.2)	82 (7.7)
	Trading	530	111 (20.9)	127 (23.9)	28 (5.3)
	Employed	87	18 (20.7)	20 (22.9)	8 (9.2)
	Student	134	15 (11.2)	19 (6.7)	6 (4.5)
Ecological zone	Western zone	461	44 (9.5)	171 (37.1)	26 (5.6)
	North-eastern	465	133 (28.6)	105 (22.6)	25 (5.4)
	Central	401	55 (13.7)	62 (15.5)	26 (6.5)
	Southern Highlands	265	31 (11.7)	73 (27.6)	23 (8.7)
	Lake Victoria	226	29 (12.8)	98 (43.4)	24 (10.6)
Sampling setting	Facility	833	117 (14.1)	243 (29.2)	65 (7.8)
	Household	985	175 (17.8)	266 (27.0)	59 (5.9)
Fever	Yes	71	9 (12.7)	34 (47.9)	10 (14.1)
	No	1747	62 (3.6)	475 (27.2)	114 (6.5)
Mosquito bite	Yes	1205	197 (16.4)	358 (29.7)	78 (6.5)
	No	613	94 (15.3)	151 (24.6)	46 (7.5)
Stagnant water	Yes	146	45 (30.8)	45 (30.8)	12 (8.2)
	No	1672	247 (14.8)	464 (27.8)	112 (6.7)
Piped water	Yes	523	113 (21.6)	155 (29.6)	35 (6.7)
	No	1295	179 (13.8)	354 (27.3)	89 (6.9)
Mosquito net use	Yes	1140	197 (17.3	360 (31.6)	71 (6.2)
	No	678	95 (14.0)	149 (21.9)	53 (7.8)
Visiting mines	Yes	35	4 (11.4)	8 (22.9)	6 (17.1)
	No	1783	288 (16.2)	501 (28.1)	118 (6.6)

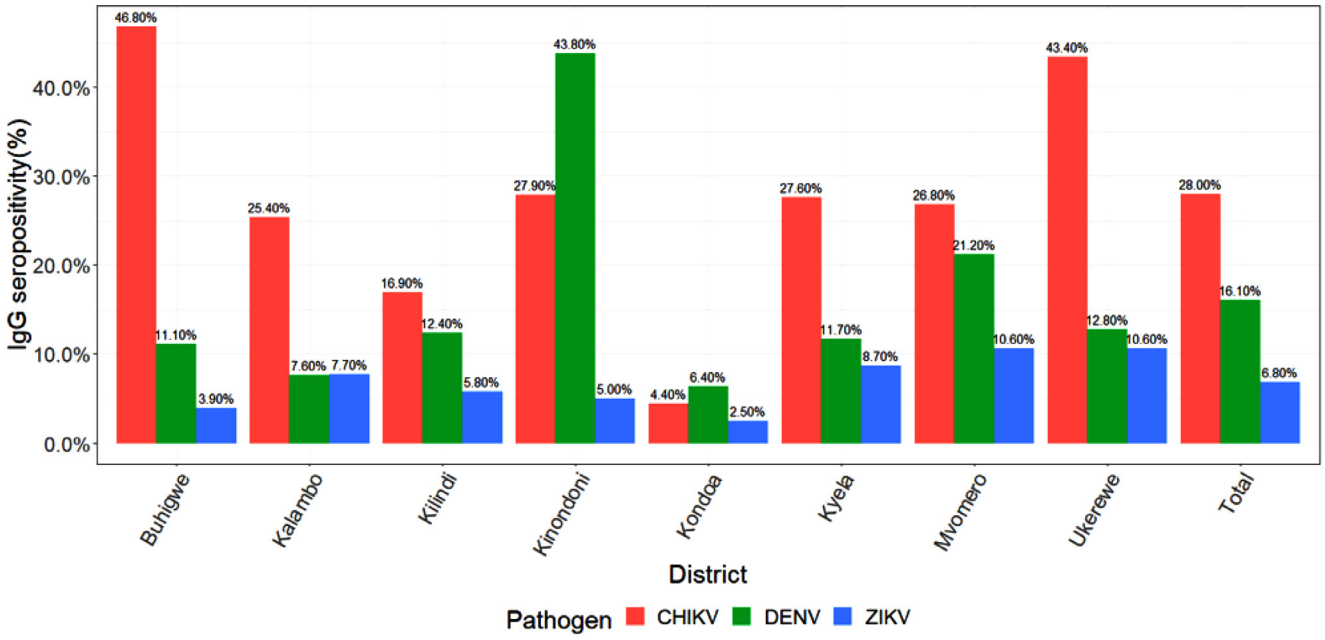

Fig. 2. Seroprevalence of immunoglobulin G (IgG) antibodies specific for dengue virus (DENV), chikungunya virus (CHIKV), and Zika virus (ZIKV) by district.

seropositivity, with individuals aged 28–42 and >42 years having been more exposed than those aged <28 years (Table 2).

Seroprevalence of co-circulation of CHIKV, DENV, and ZIKV

Of the 1818 serum samples tested, 80 (4.4%) were positive for both DENV and CHIKV IgG antibodies, 14 (0.8%) for CHIKV and ZIKV IgG antibodies, 16 (0.88%) for DENV and ZIKV IgG antibodies, and 16 (0.99%) for DENV and CHIKV and ZIKV IgG antibodies (Fig. 3). The prevalence of IgG antibodies for CHIKV+ZIKV exposure was highest in Ukerewe (4.0%; n = 224), while the highest prevalence of DENV+CHIKV antibodies was in Kinondoni (15.0%; n = 235). The overall prevalence of IgG antibodies specific to at least one pathogen (DENV or CHIKV or ZIKV) was 41% (n = 747),

Table 2
Prevalence of IgG antibodies to multiple arboviruses by sociodemographic characteristics, ecological zones, and exposure risk factors

Exposure risk factor	Category	Number tested	DENV+CHIKV+ZIKV Number IgG-positive (%)	DENV+CHIKV Number IgG-positive (%)	CHIKV+ZIKV Number IgG-positive (%)	Cross-reactivity Number IgG-positive (%)
Sex	Female	985	5 (0.5)	59 (5.9)	18 (1.8)	8 (0.9)
	Male	825	10 (1.2)	52 (6.3)	12 (1.5)	8 (0.9)
Age (years)	<28	642	1 (0.2)	15 (2.3)	8 (1.3)	6 (0.9)
	28–42	607	5 (0.8)	47 (7.7)	11 (1.8)	7 (1.2)
	>42	569	9 (1.6)	49 (8.6)	11 (1.9)	3 (0.5)
Education	Primary	1135	9 (0.8)	73 (6.4)	20 (1.8)	9 (0.8)
	Secondary	219	2 (0.9)	14 (6.4)	3 (1.4)	2 (0.9)
	Post-secondary	53	0 (0.0)	1 (1.9)	2 (3.8)	1 (1.9)
	None	411	4 (0.9)	23 (5.6)	5 (1.2)	4 (0.9)
Occupation	Farming	1067	11 (1.0)	64 (5.9)	21 (1.9)	9 (0.8)
	Trading	530	3 (0.6)	40 (7.6)	4 (0.8)	6 (1.1)
	Employed	87	0 (0.0)	5 (5.8)	4 (4.6)	1 (1.2)
	Student	134	1 (0.8)	2 (1.5)	1 (0.8)	0 (0.0)
Ecological zone	Western	461	2 (0.4)	25 (0.4)	8 (1.7)	2 (0.4)
	North-eastern	465	1 (0.2)	36 (7.7)	3 (0.7)	2 (0.4)
	Central	401	5 (1.3)	20 (4.9)	6 (1.5)	6 (1.5)
	Southern	265	2 (0.8)	16 (6.0)	3 (1.1)	4 (1.5)
	Lake Victoria	226	5 (2.2)	14 (6.2)	10 (4.4)	2 (0.9)
Sampling setting	Facility	833	6 (0.7)	47 (5.6)	20 (2.4)	6 (0.7)
	Household	985	9 (0.9)	64 (6.5)	10 (1.0)	10 (1.0)
Fever	Yes	71	2 (2.8)	5 (7.0)	1 (1.4)	0 (0.0)
	No	1747	13 (0.7)	106 (6.1)	29 (1.7)	16 (0.9)
Mosquito net use	Yes	1140	12 (1.1)	85 (7.5)	18 (1.6)	10 (0.9)
	No	678	3 (0.4)	26 (3.8)	12 (1.8)	6 (0.9)
Mosquito bite	Yes	1205	12 (0.2)	82 (6.7)	20 (1.7)	10 (0.8)
	No	613	3 (0.5)	29 (1.5)	10 (1.6)	6 (0.9)
Stagnant water	Yes	146	3 (2.1)	16 (10.9)	3 (2.1)	2 (1.4)
	No	1672	12 (0.7)	95 (5.6)	27 (1.6)	14 (0.8)
Piped water	Yes	523	5 (0.9)	62 (11.9)	9 (1.7)	7 (1.3)
	No	1295	10 (0.8)	49 (3.8)	21 (1.6)	9 (0.7)
Visiting mines	Yes	35	0 (0.0)	2 (5.7)	2 (5.7)	0 (0.0)
	No	1783	15 (0.8)	109 (6.1)	28 (1.6)	16 (0.9)

CHIKV, chikungunya virus; DENV, dengue virus; ZIKV, Zika virus.

and it was highest in Kinondoni (59.0%; $n = 255$), followed by Ukerewe (51%, $n = 224$) and Buhigwe (50%, $n = 255$) (Fig. 4). There was a significant difference in CHIKV+ZIKV seropositivity between ecological zones ($P = 0.01$), with the highest seroprevalence in the Lake Victoria zone (4.0%; $n = 224$). Individuals aged >42 years were more significantly associated with increased DENV+CHIKV (8.6%; $P < 0.05$) and DENV+CHIKV+ZIKV (1.6%; $P = 0.02$) seropositivity than their younger counterparts. Occupation ($P = 0.04$) and sampling setting ($P = 0.03$) were significantly associated with CHIKV+ZIKV seropositivity. The highest seroprevalence was among those employed (4.6%) and those sampled from health facility settings (2.4%). Having piped water ($P < 0.001$) and the presence of stagnant water around the home ($P = 0.02$) were significantly associated with DENV+CHIKV seropositivity.

Potential risk factors for DENV, CHIKV, and ZIKV seropositivity

Potential risk factors varied between the three infections, and the magnitude of the risk was found to differ significantly among the ecological zones. Age was an important factor for DENV and CHIKV seropositivity, with older age (>28 years) indicating a higher risk than in the younger population ($P < 0.001$). Experiencing frequent mosquito bites ($P < 0.01$), having piped water ($P < 0.01$), and the presence of stagnant water around the home ($P < 0.01$) were significantly associated with higher odds of DENV seropositivity, but were not important factors for CHIKV or ZIKV (Table 3). Having a fever was significantly associated with increased odds of CHIKV seropositivity ($P < 0.001$), while the use of mosquito nets was associated with higher odds for CHIKV. On the other hand, visiting mines had higher odds of ZIKV seropositivity ($P < 0.05$). In the ecological zones, the highest risks were observed for CHIKV seropositivity, with over three-fold differences in the Lake Victoria and Western zones compared to the Central zone. The North-eastern zone had higher odds of DENV seropositivity rates ($P < 0.001$) compared to the other ecological zones. A higher risk of ZIKV was identified in the Lake Victoria zone compared to the other ecological zones. However, these results were not statistically significant. Although with no statistical significance, the risk for ZIKV was estimated to be higher in the Lake Victoria zone than in the other ecological zones (Table 3).

Potential risk factors for multiple DENV, CHIKV, and ZIKV infections

Univariate and multivariable analyses showed that older age (>28 years) ($P < 0.001$) and having piped water ($P < 0.01$) were significantly associated with higher odds of DENV and CHIKV seropositivity. Fever was significantly associated with an infection of either DENV or CHIKV or ZIKV ($P < 0.001$). Individuals sampled from health facilities were significantly associated with higher odds of CHIKV+ZIKV seropositivity ($P = 0.03$) (Table 4). Residents of Lake Victoria zone were significantly at higher risk of exposure to all three pathogens (DENV+CHIKV+ZIKV) than residents of the other zones ($P < 0.05$) (Table 5).

Discussion

Chikungunya, dengue, and Zika viruses are closely related mosquito-borne viruses with similar transmission cycles, vectors, and disease manifestations. The findings of this study indicate that the three infections are prevalent across Tanzania. Overall, the seroprevalence was higher for CHIKV than DENV or ZIKV, and it varied between districts and ecological zones. CHIKV infection was most prevalent in the Western zone, DENV in the North-eastern zone, and ZIKV in the Central zone. The lowest seropositivity rates

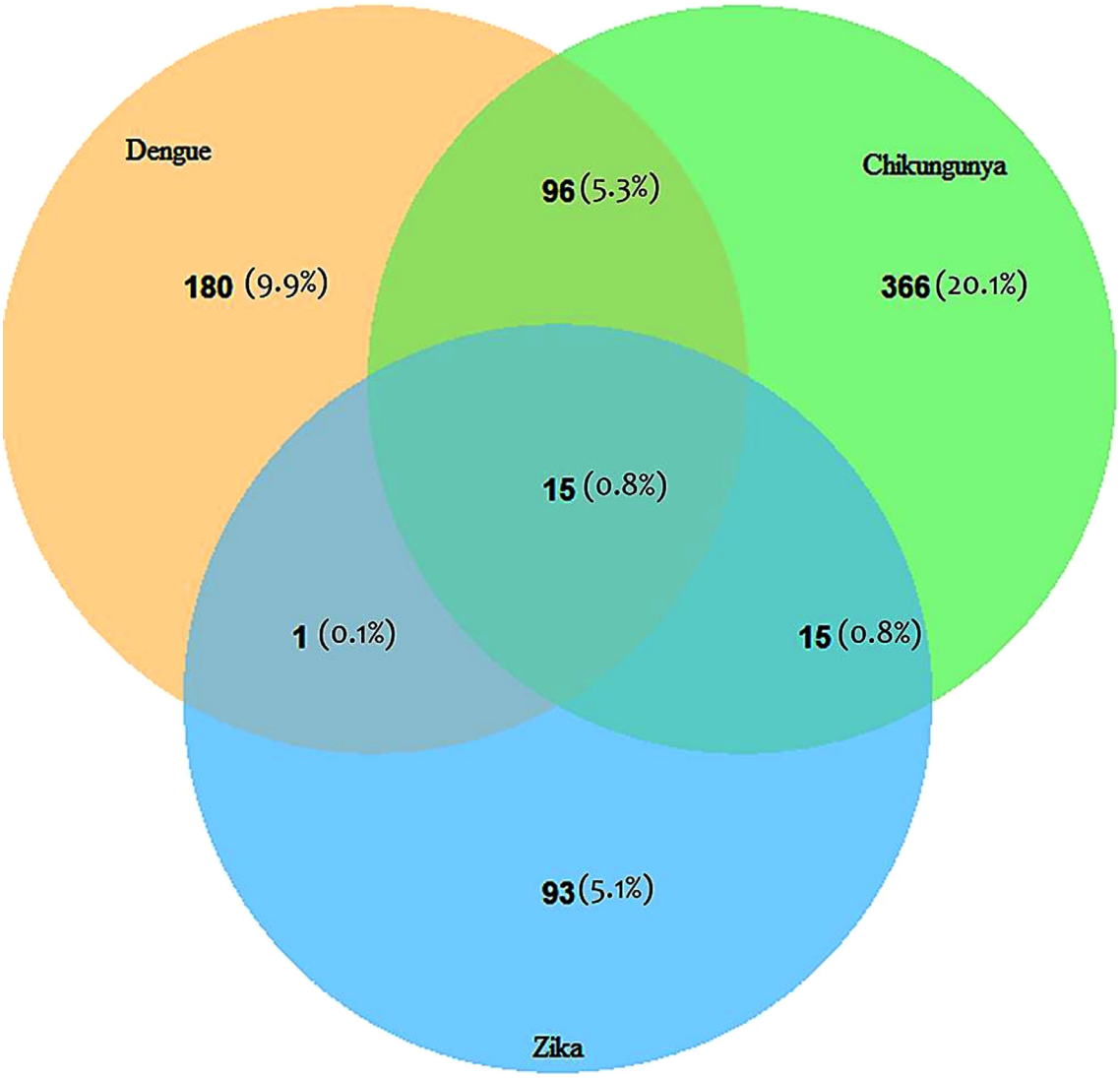

Fig. 3. Number and percentage (%) of samples positive for immunoglobulin G (IgG) antibodies to single and multiple exposures of dengue virus, chikungunya virus, and Zika virus.

of the three arbovirus infections were observed in the semi-arid district of the Central zone. In Tanzania during the past decade, dengue to a large extent, and chikungunya to a lesser extent, have been reported as important causes of morbidity, mainly in the North-eastern zone (Hertz et al., 2012; Chipwaza et al., 2014; Vairo et al., 2016). The findings of the current study highlight the presence of the three arboviruses among human populations in almost all zones of Tanzania and indicate a wide circulation of the viruses among asymptomatic individuals, hence unlikely to be diagnosed through the routine health service delivery system.

In Tanzania, several studies have reported the seroprevalence of CHIKV in different parts of the country. In the present study, the highest CHIKV seroprevalence was found in Buhigwe in the Western zone and Lake Victoria zone. Previous studies in Tanzania have reported between 1.0% and 29.3% CHIKV seroprevalence, with lower prevalence in the Central and Southern Highlands regions (Chipwaza et al., 2014; Ndosi et al., 2016; Budodo et al., 2020) and relatively higher prevalence in the Northern-eastern and Lake Victoria zones of the country (Kajeguka et al., 2016; Kinimi et al., 2018).

Similarly to CHIKV, some studies have reported the seroprevalence of DENV in different parts of the country (Vairo et al., 2012, 2016; Hertz et al., 2012; Chipwaza et al., 2020). However, most of the studies were facility-based, focusing on febrile patients (Hertz et al., 2012; Kajeguka et al., 2016; Chipwaza et al., 2020). The current study included both individuals seeking care at health facilities and those found at home. The findings showed that the prevalence of DENV antibodies was higher among those sampled in the household setting than those sampled at health facilities. Previous studies have reported higher prevalence rates of DENV infections among febrile patients in Kilosa, Kinondoni, and Ilala districts, which are located in the Central and North-eastern zones (Vairo et al., 2016; Chipwaza et al., 2020). On the other hand, relatively lower DENV prevalence in Tanzania has been reported among febrile patients in Temeke, Moshi, Iringa, Kilombero, Pemba, and Babati, indicating spatial variations in these infections (Hertz et al., 2012; Vairo et al., 2012, 2016; Faustine et al., 2017; Chipwaza et al., 2020). In this study, ZIKV was most prevalent in Mvomero district, in Central Tanzania. Studies elsewhere in Africa have reported a slightly higher prevalence of ZIKV in Senegal, but a lower prevalence in Cameroon, The Gambia, and Mali (Marchi et al., 2020; Nguyen et al., 2020).

Adults accounted for the majority of those with CHIKV infections. The tendency of the arboviruses to affect older populations

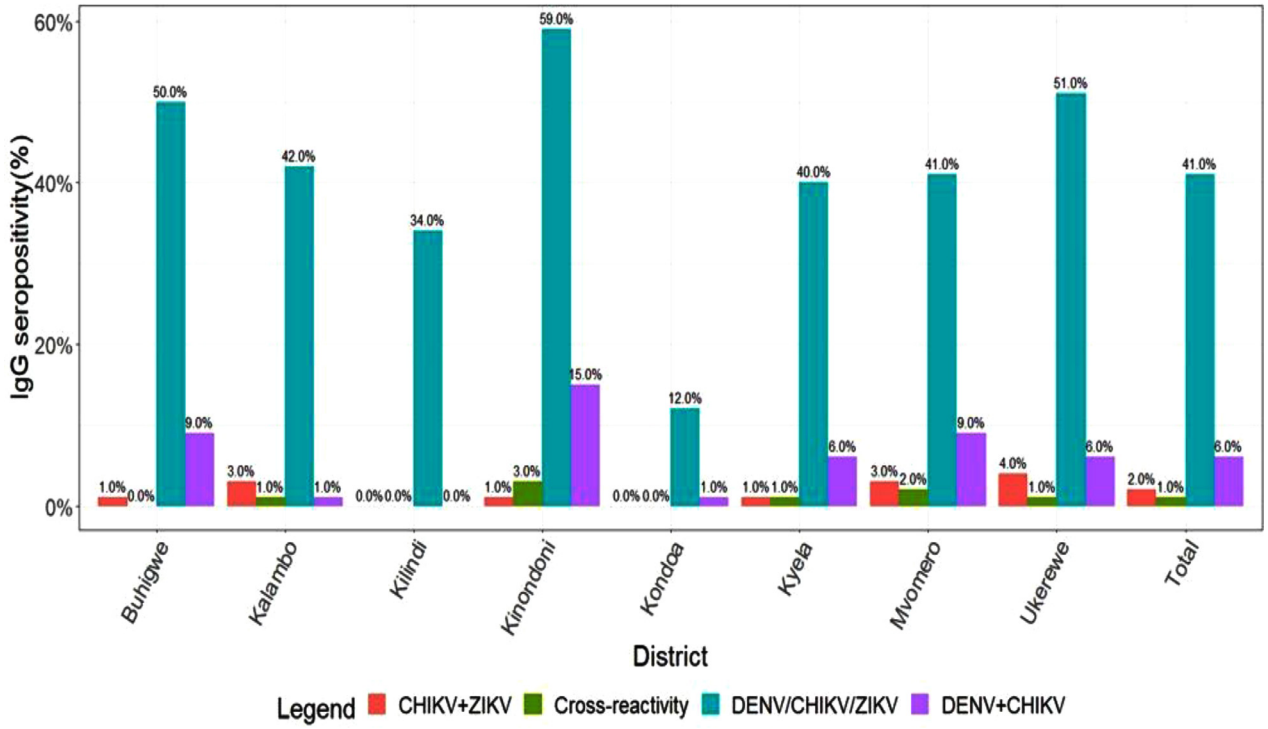

Fig. 4. Immunoglobulin G (IgG) seropositivity for multiple exposures and cross-reactivity of dengue, chikungunya and Zika viruses by district.

Table 3
Risk factors associated with dengue, chikungunya, and Zika IgG seropositivity in the univariate and multivariable logistic regression models

Exposure risk factor	Category	Dengue		Chikungunya		Zika	
		Univariate OR (95% CI)	Multivariate OR (95% CI)	Univariate OR (95% CI)	Multivariable OR (95% CI)	Univariate OR (95% CI)	Multivariable OR (95% CI)
Age (years)	<28	Ref.	Ref.	Ref.	Ref.	Ref.	-
	28–42	1.8 (1.3–2.4)***	1.9 (1.2–2.7)***	1.7 (1.3–2.2)***	1.6 (1.2–2.1)**	1.3 (0.8–1.9)	-
	>42	1.9 (1.4–2.7)***	2.3 (1.6–3.4)***	2.2 (1.7–2.9)***	2.1 (1.6–2.8)***	1.2 (0.7–1.8)	-
Occupation	Employed	Ref.	Ref.	Ref.	-	Ref.	-
	Farming	o.6 (0.4–1.1)	-	1.6 (0.9–2.7)	-	0.8 (0.4–1.9)	-
	Trading	1.0 (0.6–1.8)	-	1.1 (0.6–1.9)	-	0.6 (0.3–1.3)	-
	Student	0.5 (0.2–1.0)	-	0.6 (0.3–1.1)	-	0.5 (0.2–1.4)	-
Education	Primary	1.2 (0.9–1.7)	1.0 (0.7–1.4)	0.9 (0.8–1.3)	-	0.9 (0.6–1.4)	-
	Secondary	1.9 (1.2–2.9)**	1.4 (0.9–2.3)	0.8 (0.5–1.1)	-	1.0 (0.5–1.9)	-
	Post-secondary	0.7 (0.2–1.6)	0.4 (0.1–1.0)	0.6 (0.3–1.1)	-	1.7 (0.6–4.0)	-
	None	Ref.	Ref.	Ref.	-	Ref.	-
Ecological zone	Central	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.
	Western	0.6 (0.4–1.0)	0.7 (0.5–1.1)	3.2 (2.3–4.5)***	3.3 (2.4–4.7)***	0.9 (0.5–1.5)	0.9 (0.5–1.6)
	North-eastern	2.5 (1.8–3.6)***	2.2 (1.5–3.2)***	1.6 (1.1–2.3)**	1.7 (1.2–2.4)**	0.8 (0.5–1.5)	0.8 (0.5–1.6)
	Southern Highlands	0.8 (0.5–1.3)	0.9 (0.6–1.6)	2.1 (1.4–3.1)***	2.2 (1.5–3.3)***	1.4 (0.8–2.5)	1.3 (0.7–2.4)
	Lake Victoria	0.9 (0.6–1.5)	1.1 (0.7–1.8)	4.2 (2.9–6.1)***	4.3 (2.9–6.3)***	1.7 (0.9–3.1)	1.7 (0.9–2.9)
Fever	Yes	0.8 (0.4–1.5)	-	2.5 (1.5–3.9)***	2.4 (1.5–4.1)***	2.4 (1.1–4.5)*	2.1 (0.9–4.1)
Mosquito bites	Yes	1.7 (1.3–2.3)***	1.4 (1.1–1.9)**	1.3 (1.0–1.6)	-	0.9 (0.6–1.3)	-
Stagnant water around home	Yes	2.6 (1.8–3.7)***	1.8 (1.2–2.6)**	1.2 (0.8–1.7)	-	1.3 (0.6–2.2)	-
Piped water	Yes	1.7 (1.3–2.2)***	1.4 (1.1–1.9)**	1.1 (0.9–1.4)	-	0.9 (0.6–1.4)	-
Mosquito net use	Yes	1.3 (0.9–1.7)	-	1.6 (1.3–2.1)***	1.6 (1.3–2.0)***	0.8 (0.5–1.1)	-
Visiting mines	Yes	0.7 (0.2–1.7)	-	0.8 (0.3–1.6)	-	2.9 (1.1–6.7)**	3.2 (1.2–7.6)*

OR, odds ratio; CI, confidence interval. ***$P < 0.001$; **$P < 0.01$; *$P < 0.05$; Ref. = reference group (OR = 1).

has been reported in other studies. In a recent study in Vietnam, the prevalence of recent ZIKV infection was highest in the 46–60 years age group (Nguyen et al., 2020). Similarly, higher DENV prevalence rates were observed among older than younger individuals, an observation that corresponds with the findings of studies in Malaysia (Dhanoa et al., 2018) and elsewhere in Africa (Mwanyika et al., 2021). A low DENV prevalence among individuals <15 years old has also been reported recently in Cameroon (Tchuandom et al., 2019). As in the current study, the association of age with DENV infection in Zambia showed that those aged <5 years expressed a lower risk of DENV seropositivity than those aged ≥45 years (Mazaba-Liwewe et al., 2014). Like in CHIKV and DENV, higher ZIKV prevalence is frequent among the older individuals. In a study in Vietnam, the prevalence of ZIKV infections was highest in the 46–60 years age group (Nguyen et al., 2020), while in Brazil it was highest among those 20–45 years old (Barreto et al., 2020).

Logistic regression analysis showed higher odds of CHIKV seropositivity among individuals with fever. Chikungunya normally presents with an abrupt onset of high fever, with the majority of

Table 4
Risk factors associated with co-infection with dengue + chikungunya or chikungunya + Zika IgG in the univariate and multivariate logistic regression models

Pathogen	Exposure risk factor	Category	Univariate OR (95% CI)	Multivariable OR (95% CI)
DENV and CHIKV	Age (years)	<28	Ref.	Ref.
		28–42	3.5 (1.9–6.6)***	3.5 (1.9–6.5)***
		>42	3.9 (2.2–7.3)***	4.0 (2.3–7.5)***
	Mosquito net use	Yes	2.0 (1.3–3.2)**	1.5 (0.9–2.5)
	Mosquito bite	Yes	1.5 (0.9–2.3)	1.1 (0.7–1.8)
	Stagnant water around home	Yes	2.0 (1.1–3.5)*	1.7 (0.9–2.9)
	Piped water	Yes	2.1 (1.4–3.0)***	1.8 (1.2–2.7)**
CHIKV and ZIKV	Sampling setting	Household	Ref.	Ref.
		Facility	2.4 (1.1–5.4)**	2.7 (1.3–6.3)**
	Occupation	Employed	Ref.	Ref.
		Farming	0.4 (0.2–1.5)	0.5 (0.2–1.7)
		Trading	0.2 (0.0–0.7)**	0.2 (0.0–0.8)
		Student	0.2 (0.0–1.1)	0.2 (0.1–1.6)
	Ecological zone	Central	Ref.	Ref.
		Western	1.2 (0.4–3.6)	1.2 (0.4–3.7)
		North-eastern	0.4 (0.1–1.6)	0.5 (0.1–1.8)
		Southern Highlands	0.8 (0.2–2.9)	0.8 (0.2–3.0)
		Lake Victoria	3.1 (1.1–9.1)*	2.8 (1.1–8.5)*

OR, odds ratio; CI, confidence interval; CHIKV, chikungunya virus; DENV, dengue virus; ZIKV, Zika virus. ***$P < 0.001$; **$P < 0.01$; *$P < 0.05$; Ref. = reference group (OR = 1).

Table 5
Risk factors associated with multiple arbovirus IgG seropositivity in the univariate and multivariable logistic regression models

Pathogen	Exposure risk factor	Category	Univariate OR (95% CI)	Multivariable OR (95% CI)
DENV, CHIKV, or ZIKV	Age (years)	<28	Ref.	Ref.
		28–42	1.6 (1.2–1.9)***	1.5 (1.2–1.9)**
		>42	1.9 (1.2–2.4)***	1.9 (1.5–2.5)***
	Ecological zone	Central	Ref.	-
		Western	0.9 (0.7–1.2)	-
		North-eastern	0.7 (0.5–0.9)	-
		Southern Highlands	0.4 (0.3–0.5)	-
		Lake Victoria	1.2 (0.9–1.6)	-
	Fever	Yes	2.3 (1.4–3.8)***	2.5 (1.5–4.2)***
	Mosquito bite	Yes	1.4 (1.1–1.7)**	1.3 (1.1–1.6)*
	Stagnant water around home	Yes	1.6 (1.2–2.3)**	1.6 (1.1–2.3)**
	Piped water	Yes	1.2 (0.9–1.5)	-
	Mosquito net use	Yes	1.3 (1.1–1.6)**	1.2 (0.9–1.5)
	Visiting mines	Yes	0.9 (0.5–1.9)	-
DENV + CHIKV + ZIKV	Age (years)	<28	Ref.	Ref.
		28–42	5.3 (0.9–10)	5.4 (0.9–10)
		>42	10 (1.9–19)	9.9 (1.8–18)**
	Ecological zone	Central	Ref.	Ref.
		Western	2.0 (0.2–4.2)	1.9 (0.1–4.1)
		North-eastern	3.5 (0.3–7.6)	3.4 (0.3–7.3)
		Southern Highlands	5.9 (0.9–11)	5.5 (0.9–11)
		Lake Victoria	10 (1.7–20)*	9.9 (1.5–19)*
	Fever	Yes	3.9 (0.6–14)	-
	Mosquito bite	Yes	2.1 (0.7–9.0)	-
	Stagnant water around home	Yes	2.9 (0.7–9.3)	-
	Mosquito net use	Yes	2.4 (0.8–10)	-
	Visiting mines	Yes	0.1 (0.0–0.4)	-

OR, odds ratio; CI, confidence interval; CHIKV, chikungunya virus; DENV, dengue virus; ZIKV, Zika virus. ***P < 0.001; **P < 0.01; *P < 0.05; Ref. = reference group (OR = 1).

individuals infected developing fever, compared with DENV and ZIKV infections which are sub-clinical in the majority of infected individuals (Martinez et al., 2019). In this study, about half of those who had fever at the time of recruitment were positive for CHIKV IgG antibodies. The findings of the present study agree with the results from previous studies (Pinzón-Redondo et al., 2016; Kinimi et al., 2018). Moreover, the study results indicate that living in Kinondoni and Mvomero districts, increasing age, experiencing regular mosquito bites, piped water at home, and the presence of stagnant water around the home were potential factors associated with the risk of DENV exposure. In urban areas like Kinondoni with a high population density, solid waste disposal facilities are poor, providing conducive breeding sites for *Aedes* mosquitoes (Kholedi et al., 2012; Mukhtar et al., 2012; Camara et al., 2018). Living in Mvomero and Ukerewe districts and visiting mines were potential risk factors for ZIKV infection. The reasons for this could not be determined, although the risk for ZIKV transmission depends on the presence of vector mosquito species as a function of environmental suitability (Gardner et al., 2018).

Overall, there was a significant difference in multiple exposure to DENV, CHIKV, and ZIKV between districts. The highest DENV+CHIKV seropositivity was recorded among individuals in Kinondoni, and the highest CHIKV+ZIKV seropositivity was found in Ukerewe district. Except for CHIKV+ZIKV multiple infection, none of the individuals aged <15 years in this study had infections with two arboviruses . In this study, the mean age range of the in-

fected population was statistically higher in the patients infected with ZIKV than in those infected with DENV or CHIKV. A relatively higher prevalence of infections of the three arboviruses has been reported in a study performed at the Columbia–Venezuela border (Carrillo-Hernández et al., 2018) and among pregnant women in Mexico (Eligio-García et al., 2020). These findings demonstrate the simultaneous co-circulation of DENV, CHIKV, and ZIKV in Tanzania, especially in Kinondoni, Mvomero, and Ukerewe districts. The prevalence of co-circulation indicates the endemicity of the three arboviruses in Tanzania and emphasizes the need for molecular diagnosis to rule out serological cross-reaction and avoid false-positives (Langerak et al., 2019).

Due to high homologies of antigens within the *Flavivirus* genus, which includes DENV and ZIKV, cross-reactivity cannot be completely ruled out within the flaviviruses (Rathore and St. John, 2020). Although, multiple flavivirus infections are possible, particularly in endemic regions, in this study seropositivity to DENV+ZIKV IgG antibodies was considered as possible cross-reactivity. Given the quality of recombinant virus-specific non-structural protein 1 (NS1), the antigen source used in this study, cross-reactivity was between 1% and 3% in all study districts. Similarly, limited cross-reactivity between DENV and ZIKV has been described recently using the same NS1 ELISA kits (Steinhagen et al., 2016).

Residents of the Lake Victoria zone were significantly at higher risk of CHIKV+ZIKV co-circulation than those in the other zones. Living in Kinondoni and the presence of piped water at home were significant risk factors for DENV and CHIKV co-circulation. Living in Ukerewe, Mvomero, and Kinondoni districts were potential risk factors for DENV and ZIKV co-circulation, while living in Mvomero was a significant risk factor for CHIKV and ZIKV co-circulation. Living in Ukerewe was a potential risk factor for DENV, CHIKV, and ZIKV co-circulation. In contrast, in a study in Malaysia, no associations between arbovirus co-circulation and occupation, study site, or educational level were observed (Dhanoa et al., 2018).

Chikungunya, dengue, and Zika are thought to have originated in Tanzania (Christie, 1872; Dick et al., 1952; Robinson, 1955). Even though multiple mosquito-borne viral infections have been reported in Tanzania (Hertz et al., 2012; Chipwaza et al., 2014; Vairo et al., 2016), they have received little attention as public health threats for several decades. Co-circulation of mosquito-borne viral infections is a public health concern, because most often they cause fevers that are usually not considered by clinicians, especially in areas with inadequate laboratory capacities (Crump et al., 2013; Ayorinde et al., 2016). Thus, in many areas, malaria is over-diagnosed, and patients without malaria have had poor clinical outcomes (Crump et al., 2013). Accurate and up-to-date epidemiological data on clinical cases of these mosquito-borne viral infections are limited in many areas of sub-Saharan Africa. This is most likely because of the fact that they are asymptomatic, and when they occur, symptoms are generally mild and non-specific, and therefore may not be detected or reported from healthcare facility settings. The findings of this study provide important information for consideration in terms of the epidemiology and interventions of chikungunya, dengue, and Zika in Tanzania.

While interpreting the findings of this study, it should be noted that due to the high similarity of the target antigens within the flaviviruses, cross-reactivity cannot be ruled out. In secondary infections, IgG antibodies are expressed earlier than in primary infections; as a consequence, we are likely to have missed cases of those who presented with primary viral infections during the acute phase of infection.

In conclusion, the findings of this study provide evidence of DENV, CHIKV, and ZIKV co-circulation in diverse ecological zones of Tanzania. The widespread distribution of DENV, CHIKV, and ZIKV seroprevalence in the country emphasizes the need for more effective surveillance to monitor their occurrence and spread. It is important that surveillance and diagnostic systems for the three infections are strengthened nationwide to capture information related to arboviruses. The observed arbovirus co-circulation calls for steps to improve the differential diagnosis of febrile syndromes in order to improve clinical management and outcomes.

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Ethical approval

This study received ethical approval from the Tanzania Medical Research Coordinating Committee of the National Institute for Medical Research (Ref. NIMR/HQ/R.8c/Vol 1/1168). Written informed consent was sought from study participants prior to their involvement. Written consent was sought and obtained from the parents/guardian of each participant under 18 years of age.

Conflict of interest

All authors have an interest in emerging infectious diseases. All authors declare no conflict of interest.

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