BMC Infectious Diseases

RESEARCH

Open Access

Dengue virus transmission during non-outbreak period in Dar Es Salaam, Tanzania: a cross-sectional survey

Ummul-khair Mustafa[1*], Katharina Sophia Kreppel[1,2] and Elingarami Sauli[1]

Abstract

Background Tanzania has experienced multiple dengue outbreaks between 2010 and 2019, caused by various dengue virus (DENV) strains. In 2019, there were 6917 confirmed dengue cases and 13 deaths in Tanzania. Routine diagnosis of dengue fever is unfortunately excluded, particularly during non-outbreak periods, resulting in delayed outbreak detection and control. The aim of this study was to improve early detection and control measures for DENV by investigating its circulation in human and *Aedes aegypti* (*A.aegypti*) mosquitoes during the non-outbreak periods in Dar es Salaam, Tanzania, which is an area frequently affected by dengue outbreaks.

Methods Four hundred and fifteen (415) blood samples were collected from patients attending randomly selected health facilities in five wards; Azimio, Keko, Mtoni, Mbagala and Chamazi within Temeke district. The samples were tested for DENV NS1 antigen and anti-dengue IgM and IgG antibodies by rapid test. Then, 150 out of 415 blood samples were tested for the DENV by conventional Reverse Transcriptase Polymerase Chain Reaction (RT-PCR). Two thousand two hundred and fifty (2,250) adult female *A.aegypti* mosquitoes were collected using a Prokopack aspirator and BG sentinel trap or obtained after rearing immature stages and tested, in pools of 15 for DENV by RT-PCR. Statistical Software, SPSS version 23, was used for data analysis.

Results Of the tested blood samples, 17% (71/415) were positive by NS1 antigen, 0.5% (2/415) by IgM, 0.5% (2/415) by IgG antibodies, and 0.5% (2/415) by IgM and IgG. None of the samples tested positive by DENV RT-PCR. Moreover, 3.3% (5/150) of tested mosquito pools had DENV by RT-PCR. Individuals aged between 21 and 40 years of age had increased risk of testing positive for DENV NS1 antigen, followed by those aged 5–20 years old, particularly those residing from Azimio ward, Keko ward, Mtoni ward and Mbagala ward, p-value ≤ 0.05.

Conclusion Findings from this study revealed evidence of DENV circulation during non-outbreak periods in Dar es Salaam, Tanzania. These findings underscore the importance of including testing for dengue infection in routine differential diagnoses of febrile cases, and also frequent dengue surveillance in mosquitos. This proactive approach will help early DENV outbreak detection and control in the country.

Keywords Dengue fever, *Aedes aegypti*, Vector, Prevalence, Infection rates, Non-outbreak, Dar es Salaam, Tanzania

*Correspondence:
Ummul-khair Mustafa
mustafau@nm-aist.ac.tz
[1]School of Life Sciences and Bioengineering, Nelson Mandela African Institution of Science and Technology, Arusha, Tanzania
[2]Department of Public Health, Institute of Tropical Medicine, Antwerpen, Belgium

© The Author(s) 2024. **Open Access** This article is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License, which permits any non-commercial use, sharing, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if you modified the licensed material. You do not have permission under this licence to share adapted material derived from this article or parts of it. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by-nc-nd/4.0/.

Background

Dengue fever is said to be the fastest-growing mosquito-borne viral disease in the world [1]. The disease is caused by an RNA virus in the family Flaviviridae, genus *Flavivirus* [2]. Dengue virus (DENV) has four serotypes (DENV 1–4); infection with one serotype confers long-term immunity, while subsequent infections with other serotypes increase the risk of severe disease [2–4]. The mosquito species *Aedes aegypti (A.aegypti)* circulates DENV in human populations. Unplanned urbanisation, effects of climate change, and lack of routine vector control activities increase the risk of DENV infection [5]. All these factors increase the vector population, and in turn increase the spread and maintenance of the disease.

Infection with the DENV can result in three outcomes: asymptomatic infection, a self-limiting infection with mild symptoms or a severe infection which can turn into haemorrhagic fever or dengue shock syndrome [5]. During disease onset, dengue symptoms are similar to other febrile illnesses, including malaria, influenza, measles, zika, chikungunya, and yellow fever to name but a few [6].

Dengue poses a health concern across Africa, the Americas, Eastern Mediterranean, Southeast Asia and Western Pacific and increasingly Europe [7]. Every year, dengue affects about 390 million people, while 3.9 billion remain vulnerable [5]. In the year 2023, there were 5 million dengue cases and 5000 reported deaths [8]. Of those, Africa reported 171,991 dengue cases and 753 deaths, however, insufficient diagnostic capacity and health seeking behaviour was likely the cause for underreporting of dengue cases [9, 10]. Tanzania has been among the African countries experiencing repeated dengue outbreaks since 2010 [11]. The largest outbreaks occurred in 2014, with 1018 confirmed cases and 4 deaths, and in 2019, 6917 confirmed cases and 13 deaths [12–15]. All four dengue serotypes circulate in the country [13, 16], thus presenting an increased risk for severe dengue outbreaks. The coastal Dar es Salaam region has been the most affected by all dengue outbreaks in Tanzania [13–15]. However, epidemiologic studies in Tanga [17] and Kilimanjaro [18] regions also suggested circulation of DENV during non-outbreak periods in these areas. A recent seroprevalence survey among blood donors in Dar es Salaam in 2020, reported 1% IgM and a staggering 43.5% IgG prevalence, indicating widespread past infection and active DENV circulation during non-outbreak periods [19]. To enhance the understanding on DENV circulation in Dar es Salaam region, we conducted this study during a non-outbreak period in 2023. To achieve this, we assessed the prevalence of DENV infection among febrile patients attending randomly selected health facilities in Dar es Salaam while simultaneously testing *A.aegypti* mosquitoes for DENV, which were collected within the geographical settings of recruited patients' households. The herein results serve as important baseline information to inform proactive measures and sustained intervention for controlling dengue transmission in the country.

Methods

Study setting

This study was conducted in Temeke district, in Dar es Salaam region, Tanzania (Fig. 1). Given the limited budget for this study, Temeke district was randomly selected to represent the Dar es Salaam region, which comprises five districts: Ilala, Temeke, Kinondoni, Kigamboni and Ubungo.

Temeke district has an area of 240 square kilometres [20] and a total population of 1,346,674 people, of which 655,137 are males and 691,537 females [21]. Administratively, Temeke district is divided into 23 wards and 142 sub-wards [21, 22]. Temeke district experiences a tropical climate with high temperatures between 25 °C and 35 °C throughout the year [20, 23]. The main economic activities in Temeke district are business, petty trading and industrial activities [22]. This study was conducted in five wards, namely Azimio, Mtoni, Chamazi, Mbagala and Keko.

Study design, data collection process and laboratory analysis

Part 1: prevalence of dengue among febrile patients

A health facility-based approach was used to recruit febrile patients. Samples were prospectively collected from May to July 2023. A sample size of 415 people was considered adequate based on a published formula for cross-sectional surveys [24]. The previous seroprevalence of dengue in Dar es Salaam was 43.5% IgG [19]. The assumptions for standard normal deviation were 1.96 on a 95% confidence interval, a margin of error of 0.05 and a 10% non-response rate. To obtain 415 participants, a multi-stage sampling technique was employed (Fig. 2).

Simple random sampling by lottery method was used to select one district (Temeke) out of five districts of the Dar es Salaam region. Within the Temeke district, random numbers were used to select five health facilities to participate in the study. Within the selected health facilities, the selection of the patients to participate in this study was done based on convenient sampling of patients available for doctor consultation and meeting the inclusion criteria.

The inclusion criteria for participating in the study included being at least five years old and having complaints of fever and any two of the following symptoms commonly reported among dengue patients: headache, joint pain and muscle pain, pain behind the eyes, nausea, vomiting, and hemorrhage manifestations. Excluded

Fig. 1 Map of the study area. The map was generated using ArcGIS software, version 11.1, license number EFL96303636612

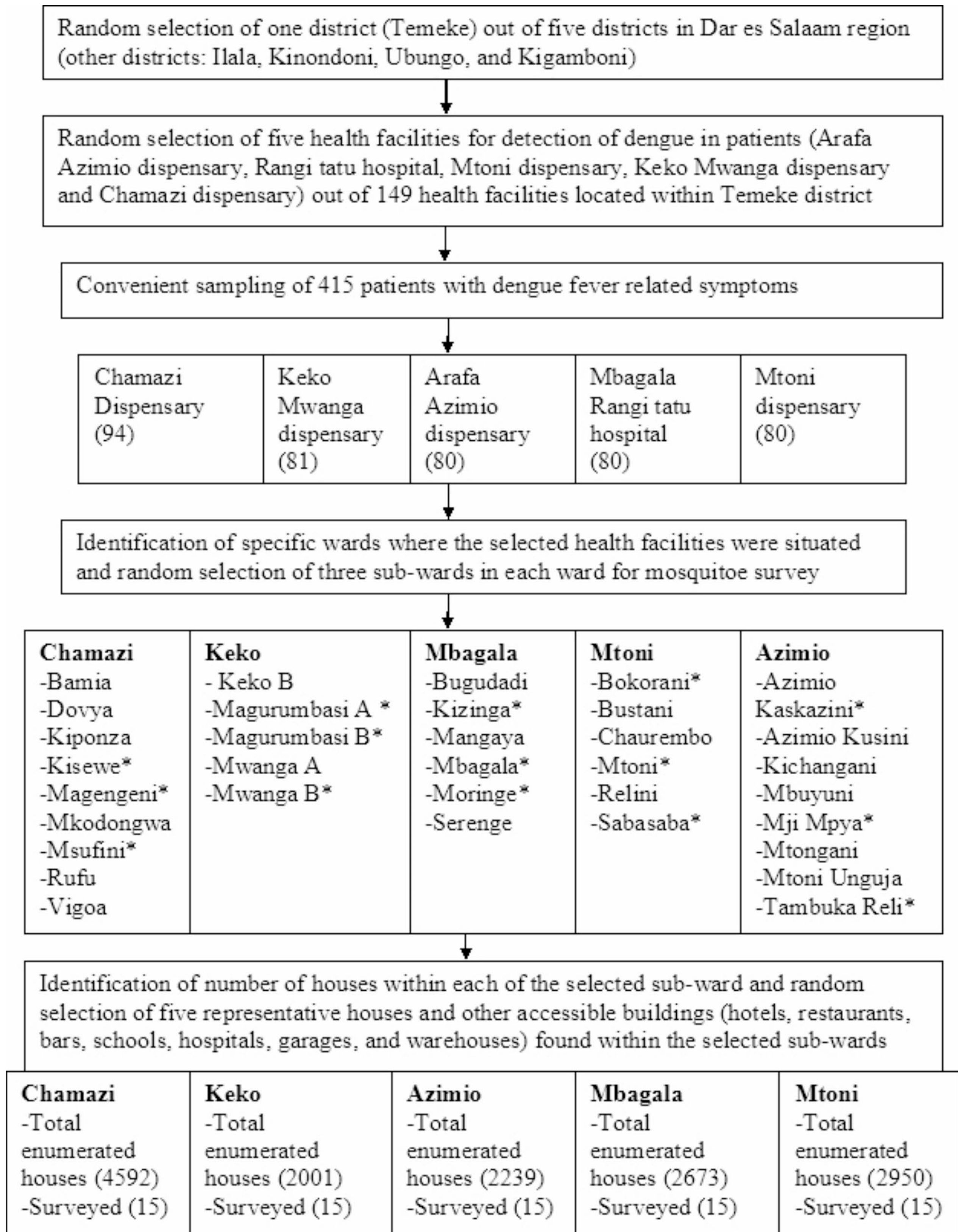

Fig. 2 Summary of sampling procedure. The asterisk sign (*) represents the selected sub-wards in each ward

from this study were patients meeting the inclusion criteria but with severe illnesses that required emergency medical care and individuals who refused to give informed consent.

Collection of blood samples

Before collecting blood samples, informed consent was obtained from all eligible participants. The protocol was approved by the Tanzania's Northern Zone Ethical Clearance Committee (Reference number: KNCHREC00061/12/2021). Patient complaints were retrieved from patient clinic cards and socio-demographic information was additionally gathered from the patients. All details were then recorded in a structured questionnaire developed specifically for this study (Additional file 1). Subsequently, 2 ml of venous blood was collected under sterile conditions into red top tubes without anticoagulant or clot activator. The blood was then centrifuged, and the resulting serum was promptly transferred into clean micro centrifuge tubes.

Detection of dengue virus antigen and antibodies

A small portion of the collected serum was immediately tested for DENV non-structural protein1(NS1) antigen, anti–dengue virus immunoglobulin M (IgM) and Immunoglobulin G (IgG) by duo dengue rapid test kit (CTK Biotech, Inc. California, United States.) [25]. As indicated in the respective test protocols, the used NS1 antigen test had sensitivity and specificity of 100% and 98.8%, respectively. The IgM test had a sensitivity and specificity of 96.9% and 98.9%, respectively. The IgG test had a sensitivity and specificity of 97.3% and 99.3%, respectively. The testing process and results were interpreted as per the manufacturer's instructions.

The remaining serum was temporarily stored at -20 °C. All samples were transported on dry ice to the Nelson Mandela African Institution of Science and Technology (NM-AIST) lab and stored at -80 °C. The samples were then shipped on dry ice to the Kilimanjaro Clinical Research Institute (KCRI) for molecular detection of DENV. A total of 150 human samples were tested for presence of the DENV genome from March to April 2024.

Detection of DENV by RT-PCR

DENV RNA was isolated using a Direct-zol™ RNA Minprep kit (Catalog No. 2053, Zymo Research Corporation, California, United States), according to the manufacturer's instructions [26]. The quality and quantity were assessed using a Thermo Scientific Nanodrop Spectrophotometer. DENV amplification from RNA templates was performed via conventional one-step reverse transcription polymerase chain reaction (RT-PCR) using the One Taq® One-step RT-PCR Kit (Catalog No. E5315S), following the standard protocol [27]. Primers used for amplification of DENV were; D1 (5'-TCAATATGCTGA AACGCGCGAGAAACCG-3') and D2 (5'-TTGCACC AACAGTCAATGTCTTCAGGTTC-3'), with 511 base pairs length [28–31]. The last step involved visualization of DENV positive samples via gel electrophoresis. The gel was stained with SYBR Green, which was bound to DNA and fluoresced under UV light, allowing visualization of the PCR products. The bands on the gel were visualized using a Gel documentation imaging system to confirm the presence of targeted DENV RNA.

Part 2: infection rates of DENV in Aedes aegypti mosquitoes

A community-based approach was used to subsequently obtain *A. aegypti* mosquitoes for determining DENV infection rates in vector populations. Samples were collected from May to July 2023.There is no standardised formula for obtaining the sample size required for the entomological survey. For this study, we collected 2,250 female *A. aegypti* mosquitoes. For the mosquito survey, a mixture of simple random sampling and convenient sampling techniques was applied, Fig. 2.

The mosquitoes were trapped from the homes of recruited patients who participated in the survey. Mosquitoes were also trapped from randomly selected houses and other accessible buildings; hotels, restaurants, bars, schools, hospitals, garages, and warehouses located within the selected study areas. For this, we first identified the geographical location of the recruited patients: Azimio, Mtoni, Keko, Mbagala, and Chamazi. Subsequently, comprehensive lists of all sub-wards within each of these wards were obtained from ward officers. Then, using a simple random sampling technique, three sub-wards were selected from each ward. Within each selected sub-ward, a thorough list of all available houses was made from the ward administrative leaders. Subsequently, five representative houses were chosen for the mosquito survey using another round of simple random sampling. Additionally, accessible public places such as hotels, restaurants, bars, schools, hospitals, garages, and warehouses within the selected sub-wards were surveyed using a convenient sampling approach.

Collection of mosquitoes

Three techniques were used to collect adult *A. aegypti* mosquitoes: BG sentinel traps, Prokopack aspirator and rearing collected larvae to adulthood. BG sentinel traps are an efficient method for collecting live, host-seeking *A. aegypti* that can be used for monitoring DENV circulation in vector populations [29]. BG sentinel traps, were placed on the ground in outdoor locations within selected houses and other accessible buildings within the surveyed wards [32]. Mosquitoes were trapped for two days, spanning twenty-four hours. Every morning, any

trapped mosquitoes were collected and transported to the National Institute for Medical Research (NIMR) for identification and storage.

A prokopack aspirator was used to collect adult mosquitoes that were resting in bushes and larger water-holding containers (car tyres and water storage tanks) found in the surveyed houses and other accessible buildings within the surveyed wards [33]. The aspiration was carried out twice in the morning and evening hours for two days.

Immature mosquito (larvae or pupae) stages were collected from artificial and natural *Aedes* breeding sites. Artificial water-holding containers ranging from soda cans to car tyres and water storage tanks form the potential breeding sites for *Aedes* mosquitoes [34]. In addition, natural water-holding containers such as tree holes, leaf axils, flower bracts and fallen leaves are *Aedes* breeding sites [34].

Standard methods for sampling immature mosquitoes were employed [35, 36]; for smaller containers, larvae and pupae were collected using a pipette, while for larger containers, larvae and pupae were collected using dippers. In case the container was too large, a bowl or small bucket was used to collect the larvae and pupae.

Collected samples of immature stages were placed in labelled glass vials with loose screw caps and then transported in cool boxes to NIMR. At NIMR, the immature mosquitoes were reared to adult mosquitoes in plastic dishes kept within netted cages at insectary environmental temperature and relative humidity of 27 ± 2 °C and 75 ± 10%, respectively [35, 36]. The larvae were fed on Whiskas® cat food biscuits, and emerging adults were fed with a 10% glucose solution in soaked cotton balls and placed at the top of the cage [37]. Emerged adult mosquitoes were collected from the cages by using a mouth aspirator and transferred to paper cups covered with mosquito nets.

Adult mosquitoes resulting from larvae/pupae rearing, as well as those directly caught by BG sentinel trap and Prokopack aspirator, were killed by freezing at -20 °C. The mosquitoes were speciated by morphological features as described before [38]. Fifteen (15) pools of *A. aegypti* female mosquitoes that were collected on the same date, in the same location and using the same trapping technique were stored in Eppendorf tubes and labelled with that information. In addition, a DNA/RNA shields (Catalog No. R1100-250, Zymo Research Corporation) was added to each tube to prevent RNA degradation [39], and all samples were stored at -80 °C at NIMR lab. The samples were shipped on dry ice to the NM-AIST lab for permanent storage until molecular analysis. Subsequently, the samples were shipped on dry ice to KCRI for molecular analysis in March 2024.

Detection of DENV in the mosquitoes

Like human samples, molecular analysis was done by conventional RT-PCR as described above. DENV RNA was isolated using a Direct-zol™ RNA Minprep kit (Catalog No. 2053, Zymo Research Corporation, California, United States), according to the manufacturer's instructions [26]. Mosquito samples were initially prepared following the additional steps specified by the manufacturer's protocol, which involved proteinase K treatment and bead beating to facilitate cell lysis and RNA release. These preparatory steps were then followed by the standard RNA extraction procedures, as provided in the kit's protocol [26] .

Statistical analysis

SPSS version 23 (IBM Corp., Armonk, NY, USA) was used for statistical analyses according to SPSS guidelines [40]. Continuous variables, including age, number of collected mosquitoes, participant gender, marital status, occupation, place of residency, and dengue test results were assessed for normality using Shapiro-Wilk and Kolmogorov-Smirnov tests.

Descriptive statistics such as mean and standard deviations (SD) and median and interquartile range (IQR) were used to summarize participants' age and mosquito count. Frequency and proportion were used to summarize participant gender, marital status, and occupation, place of residency and dengue test results in humans and mosquitoes.

Chi-square tests were initially used to compare the outcome variable, which was dengue virus NS1 positivity, across participants' socio-demographic characteristics such as age, gender, marital status, occupation and residency. Then, univariate regression analysis was conducted to assess the magnitude of association of individual predictors (independent variables) on the outcome variable. Finally, multivariate analysis was performed to account for potential confounders and to identify the combined effects of multiple variables on the outcome. For all statistical tests, a p-value ≤ 0.05 was considered significant [40].

Ethical considerations

This study received approval from Tanzania's Northern Zone Ethical Clearance Committee (Reference number: KNCHREC00061/12/2021) that included informed consent from each research participant aged 18 and above and for those under the age of 18, consent was obtained from their parents or guardians. In addition, other administrative approvals were obtained from relevant bodies.

Table 1 Socio-demographic characteristics of participants in number (%)

Variable	Azimio	Chamazi	Keko	Mbagala	Mtoni
Gender					
Female	42(16.6%)	75(29.6%)	38(15.0%)	45(17.8%)	53(20.9%)
Male	38(23.5%)	19(11.7%)	43(26.5%)	35(21.6%)	27(16.7%)
Total	80(19.3%)	94(22.7%)	81(19.5%)	80(19.3%)	80(19.3%)
Age (years)					
5–20	27(23.3%)	30(25.9%)	26(22.4%)	11(9.5%)	22(19.0%)
21–40	36(16.7%)	51(23.6%)	45(20.8%)	41(19.0%)	43(19.9%)
41–96	17(20.5%)	13(15.7%)	10(12.0%)	28(33.7%)	15(18.1%)
Total	80(19.3%)	94(22.7%)	81(19.5%)	80(19.3%)	80(19.3%)
Occupation					
Business	24(16.7%)	20(13.9%)	25(17.4%)	38(26.4%)	37(25.7%)
Employed	12(21.8%)	5(9.1%)	18(32.7%)	10(18.2%)	10(18.2%)
Farming/fishing	0(0.0%)	1(11.1%)	1(11.1%)	1(11.1%)	6(66.7%)
Students	19(18.3%)	26(25.0%)	26(25.0%)	10(9.6%)	23(22.1%)
No employment	25(24.3%)	42(40.8%)	11(10.7%)	21(20.4%)	4(3.9%)
Total	80(19.3%)	94(22.7%)	81(19.5%)	80(19.3%)	80(19.3%)
Marital status					
Married	40(22.5%)	23(12.9%)	31(17.4%)	39(21.9%)	45(25.3%)
Single	23(15.1%)	45(29.6%)	32(21.1%)	26(17.1%)	26(17.1%)
Widow	1(11.1%)	0(0.0%)	2(22.2%)	6(66.7%)	0(0.0%)
Students	16(21.1%)	26(34.2%)	16(21.1%)	9(11.8%)	9(11.8%)
Total	80(19.3%)	94(22.7%)	81(19.5%)	80(19.3%)	80(19.3%)

Table 2 Number (%) of NS1 antigen cases across participants socio-demographic features

Variables	(+)NS1 antigen	(-) NS1 antigen	X^2	Df	*P* value
Gender					
Male	38(23.5%)	124(76.5%)			
Female	33(13.0%),	220(87.0%)	7.552	1	0.006*
Age (years)					
5–20	21(18.1%)	95(81.9%)			
21–40	45(20.8%)	171(79.2%)			
41–96	5(6.0%)	78(94.0%)	9.385	2	0.009*
Occupation					
Business	27(18.8%)	117(81.2%)			
Employed	11(20.0%)	44(80.0%)			
Farming/fishing	2(22.2%)	7(77.8%)			
Students	20(19.2%)	84(80.8%)			
No employment	11(10.7%)	92(89.3%)	4.096	4	0.393
Marital status					
Married	31(17.4%)	147(82.6%)			
Single	31(20.4%)	121(79.6%)			
Widow	0(0.0%)	9(100.0%)			
Students	9(11.8%)	67(88.2%)	4.513	3	0.2111
Residency					
Azimio	19(23.8%)	61(76.2%)			
Mtoni	19(23.8%)	61(76.2%)			
Keko	19(23.5%)	62(76.5%)			
Mbagala	11(13.8%)	69(86.2%)			
Chamazi	3(3.2%)	91(96.8%)	20.753	4	0.000*

+Symbol=positive, - symbol=negative, X^{2}=chi square test value, Df=degree of freedom, *statistically significant (p-value≤0.05)

Results

Characteristics of respondents

Of the 415 febrile patients recruited for the study, 61.0% were females (n=253). The youngest patient was 5 years old while the oldest was 96 years (mean age 29.84, SD=15.58; median age 27, IQR=17). The distribution of participants in various age groups is shown in Table 1.

The participants' occupational backgrounds included, entrepreneurs, small business owners, self-employed people (144, 34.7%), employed individuals from public or private sector (55, 13.3%), farmers (9, 2.2%), primary, secondary and university students (104, 25.1%), and retired, and those actively looking for jobs and housewives 24.8% (103/415).

Marital status varied among the participants: where nearly half of them were married adults (172, 48.9%), followed by single adults (152, 36.6%), and widows (9, 2.2%). Additionally, 76 participants (18.3%) were children or students, who were generally ineligible for marriage.

The distribution of participants across different wards was nearly equal: Chamazi (94, 22.7%), Keko (81, 19.5%), Azimio (80, 19.3%), Mbagala (80, 19.3%), and Mtoni (80, 19.3%). The distribution of respondent characteristics for each ward is presented in Table 1.

Prevalence of active dengue infection by NS1 antigen rapid test

The dengue NS1 antigen was detected in 71 (17.1%) samples. The analysis of dengue NS1 antigen prevalence revealed statistically significant disparities across two demographic factors. There was a significant difference in NS1 antigen prevalence between age groups (p-value 0.009, Table 2). Prevalence of dengue was highest among participants aged 21–40 years (45/415, 20.8%), followed by participants aged 5–20 (21/415, 18.1%). Individuals aged 41–96 years had the lowest prevalence (5/415, 6.0%). Based on binary logistic regression, individuals aged between 21 and 40 years were four times more likely to be affected than participants aged 41–96 (AOR=4.321, CI=1.622–11.505, p-value=0.003), while participants aged 5–20 years were three times more likely to be NS1 positive than those aged 41–96 years (AOR=3.426,CI=1.201–9.778; p-value=0.021); see Table 3.

Place of residency emerged as a significant factor, with participants from Azimio, Mtoni and Keko wards exhibiting higher prevalence (19 cases each, 23.8%), compared to those living in Mbagala 11 (13.8%) and Chamazi wards, 3 (3.2%), p-value≤0.0001, see Table 2. Based on

Table 3 Predictors of positive NS1 antigen cases among febrile patients in Dar Es Salaam region (*N*=415)

	Univariate analysis		Multivariate analysis	
Variable	**COR[95%CI]**	**P-value**	**AOR[95%CI]**	**P-value**
Gender				
Male	2.043[1.220–3.422]	0.007*	-	-
Female	0.150(ref)			
Age (years)				
5–20	3.448[1.243–9.566]	0.017*	3.426[1.201–9.778]	0.021*
21–40	4.105[1.569–10.743]	0.004*	4.321[1.622–11.505]	0.003*
61–96	0.064(ref)		0.008(ref)	
Occupation				
Business	1.930[0.910–4.096]	0.087	-	-
Employed	2.091[0.842–5.193]	0.112	-	-
Farming/fishing	2.390[0.440-12.967]	0.313	-	-
Students	1.991[0.901–4.401]	0.089	-	-
No employment	0.120(ref)			
Marital status				
Married	1.570[0.708–3.481]	0.267	-	-
Single	1.907[0.857–4.244]	0.114	-	-
Widow	0.000[0.000]	0.999	-	-
Students	0.134(ref)			
Residency				
Azimio	9.448[2.680-33.312]	0.000*	9.184[2.557–32.992]	0.001*
Mtoni	9.448[2.680-33.312]	0.000*	9.435[2.651–33.579]	0.001*
Keko	9.296[2.638–32.761]	0.001*	7.821[2.172–28.165]	0.002*
Mbagala	4.836[1.299–18.001]	0.019*	5.194[1.362–19.792]	0.016*
Chamazi	0.033(ref)			

*Statistically significant (p-value≤0.05), COR=Crude odds ratio, AOR=Adjusted odds ratio

binary logistic regression, participants residing in Mtoni and Azimio wards were nine times more likely to be NS1 positive than those living in Chamazi ward (AOR=9.435, CI=2.651–33.579, p-value=0.001 for Mtoni, AOR=9.184, CI=2.557–32.992, p-value=0.001 for Azimio). Additionally, residents from Keko ward were seven times more likely to be NS1 positive compared to those living in Chamazi ward (AOR=7.821, CI=2.172–28.165, p-value=0.002). Also, residents of Mbagala ward were five times more likely to be NS1 positive than residents of the Chamazi ward (AOR=5.194, CI=1.362–19.792, p-value=0.016); see Table 3.

Table 4 Number (%) of adult *Aedes aegypti* mosquitoes collected by Prokopack aspirator and BG sentinel traps per ward in Temeke district, Dar Es Salaam

Variables	A. aegypti female	A. aegypti male	Total
Azimio	92(10.66%)	32(6.95%)	124(9.37%)
Mtoni	338(39.16%)	162(35.21%)	500(37.79%)
Keko	50(5.79%)	42(9.13%)	92(6.95%)
Mbagala	177(20.50%)	142(30.86%)	319(24.11%)
Chamazi	206(23.87%)	82(17.82%)	288(21.76%)
Total	863(65.23%)	460(34.76%)	1323(100.0%)

Prevalence of active dengue infection by RT-PCR

Due to highlighted resource limitations, the RT-PCR testing was conducted on 150 human serum samples (out of 415 samples) only. Of those, 71 samples tested positive for NS1, while 6 samples were positive for IgM/IgG or IgM+IgG and the remaining 73 comprised of randomly selected samples negative by rapid tests. According to obtained results, none of the samples tested positive for DENV by RT-PCR.

Prevalence of past dengue infection by IgM/IgG antibodies rapid test

Out of the 415 febrile patients tested for past dengue infection using the DENV IgM/IgG rapid test on serum samples, two (0.5%) tested positive for DENV IgM antibodies. At the same time, an equal number also tested positive for DENV IgG antibodies. Two participants (0.5%) tested positive for both DENV IgM and IgG antibodies. The sample size was inadequate for an evaluation of variation in IgM/IgG test results and socio-demographic factors.

Aedes aegypti abundance and distribution

A total of 1,323 adult *A. aegypti* were collected in Temeke district by Prokopack aspirator and BG sentinel traps. Of those, 863 (65.23%) were females and the remaining 460 (34.76%) were males. The number of mosquitoes collected per ward ranged from 93 to 500, with an average count of 264 (SD=164.41). A great proportion of these mosquitoes were collected in Mtoni ward (500, 37.79%), followed by Mbagala ward (319, 24.11%), then Chamazi ward (288, 21.76%) and finally Azimio (124, 9.37%) and Keko (92, 6.95%) wards (Table 4).

An additional 5043 *A. aegypti* adults were obtained after rearing larval/pupal stages collected from *Aedes* breeding sites. Of those, 2728 (54.09%) were females, and the remaining 2315 (45.9%) were male. The number of adult mosquitoes reared from larvae per ward ranged from 452 to 1760, with an average count of 1008.6 (SD=528.42). The highest percentages were obtained from Mbagala ward (1,760, 34.89%) and Mtoni ward (1310, 25.97%), followed by Chamazi ward (880, 17.44%)

and lastly Azimio (641, 12.71%) and Keko (452, 8.96%) wards (Table 5).

The larvae and pupae reared to obtain adult *A. aegypti* were from various breeding sites. As shown in Table 6, the most common breeding sites were car tyres 325(68.90%), followed by discarded metal cans 37 (7.83%), buckets and water storage tanks 24(5.08%), discarded plastic containers 21 (4.44%) and car bumpers/mudguard 19 (4.02%). The remaining proportion consisted of other items such as motorcycle tyres, helmets, toilet seats, plastic television components and shoes.

The number of breeding sites per ward ranged from 28 to 153, with an average count of 94.4 (SD=56.65). The highest percentages were obtained from Mtoni ward (153, 32.41%), followed by Chamazi ward (143, 30.29%), then Mbagala ward (104, 22.03%), and the remaining were found in Azimio ward (44, 9.32%) and Keko ward (28, 5.93%), see Table 6. A visual representation of some of the breeding sites found in the study areas is shown in Fig. 3.

Table 5 Number (%) of adult *Aedes aegypti* mosquitoes obtained from larvae/pupae rearing technique by wards in Temeke district, Dar Es Salaam

Variables	A. aegypti female	A. aegypti male	Total
Azimio	356(13.04%)	285(12.31%)	641(12.71%)
Mtoni	550(20.16%)	760(32.82%)	1,310(25.97%)
Keko	392(14.36%)	60(2.59%)	452(8.96%)
Mbagala	930(34.09%)	830(35.85%)	1,760(34.89%)
Chamazi	500(18.32%)	380(16.41%)	880(17.44%)
Total	2728(54.09%)	2315(45.90%)	5,043(100.0%)

Proportion of Aedes aegypti pools positive for DENV

RT-PCR was conducted on a total of 2,250 female *A.aegypti*, which were divided in 150 pools. Among those, 55(36.66%) pools were created from female *A.aegypti* collected through the Prokopack aspirator and BG sentinel trap, and the remaining 95(63.33%) pools were created from randomly sampled female *A.aegypti* obtained after raring larvae and pupa stages. Each pool comprised 15 individual mosquitoes collected at the same location.

The RT-PCR testing confirmed active infection of DENV in five *A.aegypti* pools (5/150, 3.3%), and all positive samples were collected from the Azimio ward. Only the pools derived from the captured adults tested positive for the DENV. Table 7 provides a summary of the number of female *A.aegypti* mosquito pools tested for DENV and proportion of positive pools. Figure 4 provides a visual representation of gel electrophoresis results for some of the tested mosquito samples.

Discussion

To the best of our knowledge, this is the first study to simultaneously investigate DENV transmission in both human and mosquito vectors collected within the geographical settings of recruited patients' households during non-outbreak periods in the Dar es Salaam region of Tanzania. This allows interrogation of human and vector infections, thus allowing for more robust epidemiological evidence of presence or absence of DENV circulation during non-outbreak periods.

Seventeen per cent (71/415) of the tested human blood samples were positive by NS1 antigen test, indicating active dengue infection, even though all human samples were negative for DENV by RT-PCR. This finding may be explained by the fact that, the RT-PCR test is more specific than the NS1 antigen test [41, 42]. We speculate that, the patients did not seek medical care soon enough as has been described before [43–45]. In the context of dengue fever, 5 days after fever onset, the DENV genome is often already cleared and becomes undetectable by RT-PCR [4, 6, 41, 42, 46, 47]. However, the NS1 has a longer

Table 6 Types of water holding containers infested with *Aedes Aegypti* larvae and pupae by ward in Temeke district, Dar Es Salaam

Variables	Azimio	Mtoni	Keko	Mbagala	Chamazi	Total
Tree holes	0(0.0%)	1(0.65%)	0(0.0%)	0(0.0%)	2(1.39%)	3(0.63%)
Wells	0(0.0%)	2(1.30%)	0(0.0%)	0(0.0%)	0(0.0%)	2(0.42%)
Buckets/ tanks	3(6.81%)	1(0.65%)	0(0.0%)	16(15.38%)	4(2.79%)	24(5.08%)
Flower pots	0(0.0%)	8(5.22%)	0(0.0%)	0(0.0%)	0(0.0%)	8(1.69%)
Metal cans	0(0.0%)	8(5.22%)	7(25.0%)	0(0.0%)	22(15.38%)	37(7.83%)
Plastic containers	2(4.54%)	4(2.61%)	5(17.85%)	10(9.61%)	0(0.0%)	21(4.44%)
Plastic bags	0(0.0%)	0(0.0%)	2(7.14%)	6(5.76%)	0(0.0%)	8(1.69%)
Car tires	37(84.09)	124(81.04%)	14(50.0%)	57(54.80%)	93(65.03%)	325(68.9%)
Car bumpers	0(0.0%)	0(0.0%)	0(0.0%)	15(14.42%)	0(0.0%)	19(4.02%)
Motorcycle tires	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	2(1.39%)	2(0.42%)
Helmet	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	3(2.09%)	3(0.63%)
Toilet seats	1(2.27%)	1(0.65%)	0(0.0%)	0(0.0%)	7(4.89%)	9(1.90%)
Television component	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	10(6.99%)	10(2.11%)
Shoes	1(2.27%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	1(0.21%)
Total	44(9.32%)	153(32.41%)	28(5.93%)	104(22.03%)	143(30.29%)	472(100%)

Fig. 3 Sample breeding sites found in surveyed wards in Temeke district Dar es Salaam, Tanzania

detection window of up to 9 days after fever onset and individuals who tested negative for the DENV by RT-PCR may still test positive for the NS1 antigen [4, 6, 41, 42, 46, 47], which might have been the case in our findings.

No studies had previously investigated DENV NS1 antigen during non-outbreak period in Tanzania [17, 19, 48–51], making comparison between our findings and previous studies difficult. Moreover, we found 17% (71/415) prevalence of active DENV infections in this

Table 7 Number of *Aedes aegypti* mosquito pools tested and their positivity for dengue virus infection by study sites in Temeke district, Dar Es Salaam, Tanzania

Variable	Pools tested by PCR	Individuals pooled by ward	Positive pools	Percentage of positive pools
Azimio	25	375	5	20.0%
Mtoni	41	615	0	0.0%
Keko	22	330	0	0.0%
Mbagala	30	450	0	0.0%
Chamazi	32	480	0	0.0%
Total	150	2,250	5	3.33%

study, which was higher than the one reported in Tanga (2.2%,8/362) [17], Manyara (1.54%,1/65) [49] and Zanzibar (6.04%,9/149) [51], during non-outbreak periods. Previous studies investigated active DENV infection only by polymerase chain reaction techniques. In our study, no cases were detected by RT-PCR, underscoring its limitations in identifying active dengue infections when viral RNA levels are low. However, incorporating NS1 antigen detection enabled us to capture 71 active DENV cases that would have otherwise gone undetected by RT-PCR. This approach underscores the importance of including NS1 test as a complementary diagnostic tool to PCR, particularly for early and sensitive detection of active dengue infections [6, 41, 42].

Furthermore, when comparing our findings to studies from other African countries conducted during non-outbreak periods, our reported prevalence of NS1 at 17% remains notably higher than prevalence reported in Mozambique,1.0% (3/305) [52], 2% (2/103) in Kenya [53], and 6.1% (59/961) in Cameroon [54]. Variability in DENV infection across studies may be due to differences in the timing of data collection, as well as varying levels of sensitivity and specificity of employed diagnostic tests.

Our investigation of DENV in mosquitoes offered additional evidence supporting circulation of the DENV within the study area. Our study found 3.3% DENV infection rates in *A.aegypti*. Based on the RT-PCR test, 5 *A.aegypti* pools out of 150 had evidence for active DENV infection. The proportion of infected mosquitoes reported in our study is higher than reported in Dar es Salaam (0%, 0/51 pools) immediately after 2019 outbreak [55], suggesting resurgence of DENV transmission among *A.aegypti* mosquitoes in the area.

Varying DENV infection rates among mosquitoes have been found across different studies in Kenya (1.3% (5/386) [56], 2.3% (6/259) in Saudi Arabia [57], 39.13% (18/46) in India [58], 47.6% (10/21) in Mwanza, Tanzania [30], and 58.54% (24/41) in Nigeria [59]. A combination of factors, including competent vector species diversity and varied risk factors for dengue endemicity in the study areas could influence the infection rates across these studies. For example, in our study, we investigated DENV infection rates in one *Aedes* species, specifically *A. aegypti.* In contrast, previous studies that reported higher DENV infection rates in mosquitoes during non-outbreak periods tested multiple *Aedes* species and identified presence of multiple competent vectors: *A. aegypti, and Aedes africanus* (*A. africanus)* in Mbeya, Tanzania [30], *A. aegypti and Aedes albopictus (A. albopictus)* in India [58], *A. aegypti*, *A. albopictus*, and *Aedes galloisi (A. galloisi)* in Nigeria [59].

The highest number of dengue cases ever across the globe was recorded in the year 2023 but no cases were reported from Tanzania [8]. Our findings suggest that, the DENV circulation in Dar es Salaam in 2023 went unrecognized/ or clinically misdiagnosed for malaria due to inadequate testing capacity and infrastructure for dengue surveillance in health facilities [8, 10, 60]. Prediction

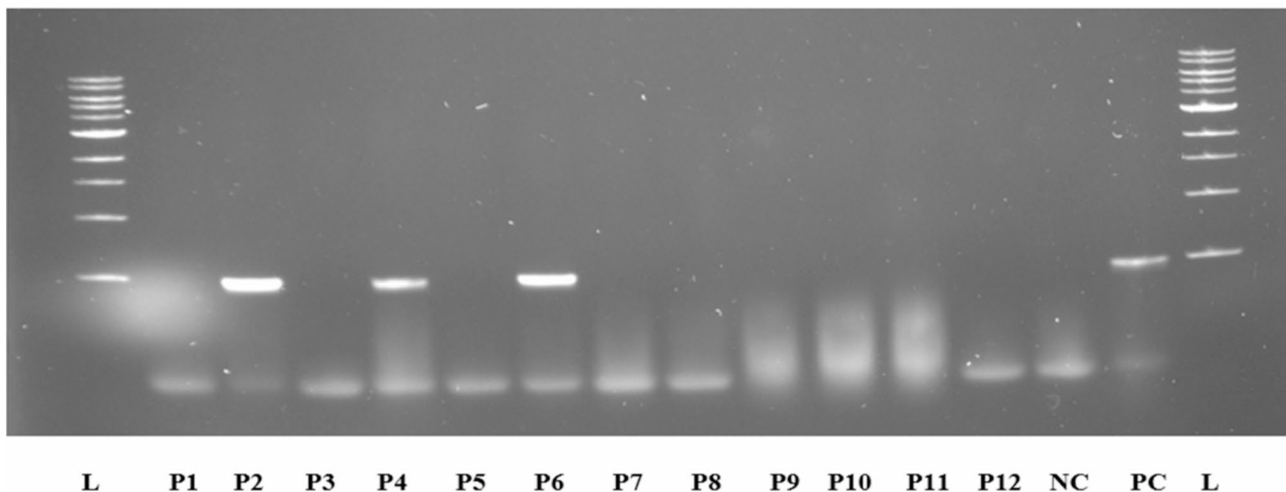

Fig. 4 Agarose gel image of RT-PCR test results in female *Aedes aegypti* mosquitoes. L is Quick-Load 1 kb DNA Ladder (NEB N0468S), P1-12 is representative of tested mosquitos' pools, NC is a negative control, and PC is a positive control. The expected band size was 511 base pairs

models published in 2016 [15] and 2020 [61] alerted to the potential for widespread and maintained DENV transmission in Tanzania due to climate change. Unfortunately, dengue remains a neglected issue in the country, leading to underreporting and misdiagnosis of patients as having malaria, other parasitic or bacterial infections [62, 63].

The finding of positive DENV cases in both human and mosquito samples collected within the same geographical settings indicates local DENV transmission within the studied Temeke district. These findings underscore the need for targeted vector control measures, such as larvae source reduction, and education efforts for raising awareness on the risk of DENV infection circulation in Dar es Salaam.

Higher risk for DENV infection was found among the younger population aged between 5 and 40 years of age compared to older individuals aged between 41 and 96. This finding may possibly imply that, Dar es Salaam region may have had a long history of dengue which allowed adult individuals to acquire age hood DENV immunity following multiple encounters with DENV. Prevalence of anti-dengue IgG antibodies in the Dar es Salaam region was approximately 43.8% (103/235) for the Kinondoni [64] district and 43.5% (44/101,43.5%) for the Temeke district [19]. Moreover, urban areas such as Azimio, Keko, Mtoni and Mbagala wards presented higher risk for dengue infection in this current study, compared to Chamazi ward, which is a semi-urban area. Similar results were reported in Zanzibar, where participants residing in urban areas were four times more likely to be exposed to the vector *Aedes* mosquito bites, and hence higher DENV infection than those living in the rural areas [50]. *Aedes* mosquitoes are highly adapted to urban man-made environments that provide abundant breeding sites, dense human populations, and heightened human interactions driven by economic activities.

Conclusion

In conclusion, our study provides evidence of established local DENV infections in humans during non-outbreak periods in Dar es Salaam, whereas previous studies reported dengue fever during outbreaks periods. The study has simultaneously shown evidence of DENV circulation in the *Aedes* vector. These findings underscore the importance of including routine dengue fever screening as part of DENV infection surveillance even during non-outbreak periods. The main limitation of this study resides in two aspects. First, due to budget limitations, we could not progress to identify specific dengue serotypes that were circulating in Temeke. The second limitation was the time gap between sample collection (both human and mosquitoes) and analysis to determine the dengue viral genome by RT-PCR. The samples were collected from May-July, 2023 and analyzed between March-April, 2024. This may have led to dengue RNA degradation. Despite these limitations, this study is the first to report on active DENV circulation during non-outbreak periods in Dar es Salaam. Our data showed presence of DENV in the humans and vectors during non-epidemic periods, making it valuable in planning and implementing effective DENV control measures.

Abbreviations

AOR	Adjusted odds ratio
A. aegypti	Aedes aegypti
A. africanus	Aedes africanus
A. albopictus	Aedes albopictus
A. galloisi	Aedes galloisi
COR	Crude odds ratio
DENV	Dengue virus
IgG	Anti–dengue virus Immunoglobulin G
IgM	Anti–dengue virus Immunoglobulin M
IQR	Interquartile range
KCRI	Kilimanjaro Clinical Research Institute
NIMR	National Institute for Medical Research
NM-AIST	Nelson Mandela African Institution of Science and Technology
NS1	Dengue virus Non-structural protein 1
P-value	Probability value
RT-PCR	Reverse Transcriptase Polymerase Chain reaction
SD	Standard deviation

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12879-024-10109-5.

Supplementary Material 1

Acknowledgements

We are grateful to Alex Limwagu (Ifakara Health Institute, Tanzania) for creating the map of the study area. We thank Demitrius Max (National Institute for Medical Research, Tanzania), Abdallah Zacharia (Muhimbili University of Health and Allied Sciences), Dr. Mgeni Mohammed, Frank Tenywa, and Iddy Mapipi (Ifakara Health Institute, Tanzania), Baraka Charles, Jesca Manyela, Edwin Lungwa, Edgar Bonajose and Mdalisi Kyambaha (Laboratory technicians of recruited health facilities) and Mr. Badi H. Badi (residency of Temeke district) for advice/support in fieldwork. We thank Dr. Happiness Kumburu, Ndealilia Swai, and Magweiga Magige (Kilimanjaro Clinical Research Institute, Tanzania), Dr. Beatus Lymo, Samson Lymo, Lydia Munuo, Niwael Kaaya and Namvua Kimera (Nelson Mandela African Institution of Science and Technology, Tanzania), Dr. Gaspary Mwanyika (Mbeya University of Science and Technology, Tanzania) and Maria Kelly (National Public Health Laboratory, Ministry of Health Tanzania) for advice/technical support in laboratory work. We further express our gratitude to the office of the district medical officer of Temeke, leaders of health facilities and the patients that were recruited in the study, leaders of selected wards and sub-wards and leaders of houses and buildings that were included in the mosquito surveillance.

Author contributions

U-k.M, K.S.K and E.S contributed to the study's conception. K.S.K and E.S contributed to funding acquisition. U-k.M and E.S contributed to data acquisition, analysis and interpretation. U-k.M and K.S.K contributed to the manuscript's conception. U-k.M drafted the manuscript. K.S.K and E.S revised the manuscript. All authors have read and approved the submitted version.

Funding

Funding was provided by the Institute of Tropical Medicine, Antwerp, as part of the Joint Pump Priming funding scheme. The funder had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Data availability
The datasets used and analysed during the current study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate
This study received ethical approval from Tanzania's Northern Zone Ethical Clearance Committee (Ref. no. KNCHREC00061/12/2021).Written informed consent was obtained from all research participants, and in the case of minors, from their parents or guardians.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Received: 4 July 2024 / Accepted: 22 October 2024
Published online: 29 October 2024

References
1. Kularatne SA, Dalugama C. Dengue infection: global importance, immunopathology and management. Clin Med. 2022;22(1):9–13. https://doi.org/10.7861/clinmed.2021-0791.
2. Kumar A, Arya H, Verma P, Singh S, Kumar V, Mittal S, et al. A review of Dengue Virus Genome, Structural and Non-structural Proteins, and Life Cycle. J Sci Innov Nat Earth. 2023;3(2):7–16. https://doi.org/10.59436/https://jsiane.com/archives3/2/76.
3. Aruna R. Review on dengue viral replication, assembly and entry into the host cells. Int J Curr Microbiol App Sci. 2014;3(11):1025–39. https://www.ijcmas.com/vol-3-11/R.Aruna.pdf.
4. Roy SK, Bhattacharjee S. Dengue virus: epidemiology, biology, and disease aetiology. Can J Microbiol. 2021;67(10):687–702. https://doi.org/10.1139/cjm-2020-0572.
5. WHO World Health Organization. Dengue and severe dengue. https://www.who.int/news-room/fact-sheets/detail/dengue-and-severe-dengue (2024). Accessed 10 May 2024.
6. Muller DA, Depelsenaire ACI, Young PR. Clinical and Laboratory diagnosis of Dengue Virus infection. JID. 2017;215(2):89–95. https://doi.org/10.1093/infdis/jiw649.
7. Brem J, Elankeswaran B, Erne D, Hedrich N, Lovey T, Marzetta V, et al. Dengue homegrown in Europe (2022 to 2023). New Microbes New Infect. 2024;56:2023–5. https://doi.org/10.1016/j.nmni.2023.101205.
8. World Health Organization. Disease outbreak news: Dengue-Global situation. https://www.who.int/emergencies/disease-outbreak-news/item/2023-DON498(2023). Accessed 10 May 2024.
9. Mustafa UK, Sauli E, Brinkel J, Kreppel KS. Health professionals' knowledge on dengue and health facility preparedness for case detection: a cross-sectional study in Dar Es Salaam, Tanzania. PLoS Negl Trop Dis. 2023;17(11):1–25. https://doi.org/10.1371/journal.pntd.0011761.
10. Mboera LEG, Sindato C, Mremi IR, Rumisha SF, George J, Ngolongolo R, et al. Socio-Ecological Systems Analysis and Health System Readiness in responding to Dengue Epidemics in Ilala and Kinondoni Districts, Tanzania. Front Trop Dis. 2021;2:1–11. https://doi.org/10.3389/fitd.2021.738758.
11. Boillat-Blanco N, Klaassen B, Mbarack Z, Samaka J, Mlaganile T, Masimba J, et al. Dengue fever in Dar Es Salaam, Tanzania: clinical features and outcome in populations of black and non-black racial category. BMC Infect Dis. 2018;18(644):1–10. https://doi.org/10.1186/s12879-018-3549-z.
12. Chipwaza B, Sumaye RD, Weisser M, Gingo W, Yeo NKW, Amrun SN, et al. Occurrence of 4 Dengue Virus Serotypes and Chikungunya Virus in Kilombero Valley, Tanzania, during the Dengue Outbreak in 2018. Open Forum Infect Dis. 2020;8(1):1–6. https://doi.org/10.1093/ofid/ofaa626.
13. Kelly ME, Msafiri F, Affara M, Gehre F, Moremi N, Mghamba J, et al. Molecular Characterisation and Phylogenetic Analysis of Dengue Fever Viruses in three outbreaks in Tanzania between 2017 and 2019. PLoS Negl Trop Dis. 2023;17(4):1–14. https://doi.org/10.1371/journal.pntd.0011289.
14. Mwanyika GO, Mboera LEG, Rugarabamu S, Sindato C, Lutwama JJ, Paweska JT, et al. Circulation of dengue serotype 1 viruses during the 2019 outbreak in Dar Es Salaam, Tanzania. Pathog Glob Health. 2021;115(7–8):1–9. https://doi.org/10.1080/20477724.2021.1905302.
15. Mweya CN, Kimera SI, Stanley G, Misinzo G, Mboera EG. Climate change influences potential distribution of infected Aedes aegypti Co-occurrence with Dengue Epidemics Risk areas in Tanzania. PLoS ONE. 2016;11(9):1–13. https://doi.org/10.1371/journal.pone.0162649.
16. Mwanyika GO, Mboera LEG, Rugarabamu S, Ngingo B, Sindato C, Lutwama JJ, et al. Dengue virus infection and associated risk factors in Africa: a systematic review and meta-analysis. Viruses. 2021;13(4):1–17. https://doi.org/10.3390/v13040536.
17. Kajeguka DC, Mponela FM, Mkumbo E, Kaaya AN, Lasway D, Kaaya RD, et al. Prevalence and Associated Factors of Dengue Virus Circulation in the Rural Community, Handeni District in Tanga, Tanzania. J Trop Med. 2023;2023:1–9. https://doi.org/10.1155/2023/5576300.
18. Kajeguka DC, Kaaya RD, Mwakalinga S, Ndossi R, Ndaro A, Chilongola JO, 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 Infect Dis. 2016;16:1–9. https://doi.org/10.1186/s12879-016-1511-5.
19. Shauri HS, Ngadaya E, Senkoro M, Buza JJ, Mfinanga S. Seroprevalence of Dengue and Chikungunya antibodies among blood donors in Dar Es Salaam and Zanzibar, Tanzania: a cross-sectional study. BMC Infect Dis. 2021;21:1–6. https://doi.org/10.1186/s12879-021-06549-y.
20. Temeke Municipal Council. Investment Profile of Temeke Municipal Council. Temeke Municipal Council. 2018. https://temekemc.go.tz/storage/app/media/uploaded-files/PROFILEYAUWEKEZAJI.pdf. Accessed 22 April 2024.
21. The United Republic of Tanzania. The 2022 Population and Housing Census: Administrative Units Population Distribution Report. Tanzania National Bureau of Statistics. 2022. https://www.nbs.go.tz/nbs/takwimu/Census2022/Administrative units Population Distribution Report Tanzania. Accessed 22 April 2024.
22. Temeke Municipal Council. Health Department Information Bureau -Temeke Municipal Council.http://www.temekemc.go.tz/storage/app/uploads/public/59d/ba8/62e/59dba862ed84d383294414.pdf. Accessed 22 April 2024.
23. Temeke Municipal Council. Temeke Municipal Council: Council Strategic Plan 2021/22 TO 2024/25. https://temekemc.go.tz/storage/app/media/uploaded-files/STRATEGICPLAN.pdf Accessed 22 April 2024.
24. Yusuf AM, Ibrahim NA. Knowledge, attitude and practice towards dengue fever prevention and associated factors among public health sector health-care professionals: in dire Dawa, eastern Ethiopia. Risk Manag Health Policy. 2019;12:91–104. https://doi.org/10.2147/RMHP.S195214.
25. CTK Biotech. The OnSite Duo Dengue Ag-IgG/IgM Rapid Test. https://ctkbiotech.com/product/duo-dengue-ag-igg-igm-rapid-test-ce/. Accessed 22 April 2024.
26. Zymo Research cooperation. Direct-zolTM RNA Minprep kit. https://files.zymoresearch.com/protocols/r2050r2051r2052r2053direct zolrnaminiprep.pdf (2023). Accessed 22 April 2024.
27. New England Biolabs Inc. One Taq ® One-Step RT-PCR Kit. https://www.neb.com/en/-/media/nebus/files/manuals/manuale5315.pdf?rev=d891c3dd27ac40269fe7 (2020). Accessed 22 April 2024.
28. Lanciotti RS, Calisher CH, Gubler DJ, Chang GJ, Vorndam AV. Rapid detection and typing of dengue viruses from clinical samples by using reverse transcriptase-polymerase chain reaction. J Clin Microbiol. 1992;30(3):545–51. https://doi.org/10.1128/jcm.30.3.545-551.1992.
29. Mboera LEG, Mweya CN, Rumisha SF, Tungu PK, Stanley G, Makange MR, et al. The risk of Dengue Virus Transmission in Dar Es Salaam, Tanzania during an epidemic period of 2014. PLoS Negl Trop Dis. 2016;10(1):1–15. https://doi.org/10.1371/journal.pntd.0004313.
30. Patrick BN, Kinimi E, Shayo MJ, Ang SO, Weyer J, Vuren PJ, Van, et al. Distribution and diversity of mosquitoes and the role of Aedes in the transmission of arboviruses in selected districts of Tanzania. Int J Mosq Res. 2018;5(1):53–60. https://www.dipterajournal.com/pdf/2018/vol5issue1/PartA/5-1-2-896.pdf.
31. Philbert A, Msonga A. Dengue vector distribution and their infection status in selected regions in Tanzania. Tanzan J Sci. 2020;46(3):636–46. https://doi.org/10.4314/tjs.v46i3.6.
32. Maciel-de-freitas R, Eiras ÁE, Lourenço-de-oliveira R. Field evaluation of effectiveness of the BG-Sentinel, a new trap for capturing adult Aedes aegypti (Diptera: Culicidae). Mem Inst Oswaldo Cruz Rio De Janeiro. 2006;101(3):321–5. https://doi.org/10.1590/S0074-02762006000300017.
33. Maia MF, Robinson A, John A, Mgando J, Simfukwe E, Moore SJ. Comparison of the CDC Backpack aspirator and the Prokopack aspirator for sampling

indoor- and outdoor-resting mosquitoes in southern Tanzania. Parasit Vectors. 2011;4:1–10. https://doi.org/10.1186/1756-3305-4-124.
34. Philbert A, Ijumba JN. Preferred breeding habitats of Aedes Aegypti (Diptera-Culicidae) Mosquito and its public health implications in Dares Salaam, Tanzania. E3. J Environ Res Manage. 2013;4(10):344–51. http://www.e3journals.org.
35. Kampango A, Furu P, Sarath DL, Haji KA, konradsen F et al. S c h i øl e r KL,. Targeted elimination of species-rich larval habitats can rapidly collapse arbovirus vector mosquito populations at hotel compounds in Zanzibar. Med Vet Entomol. 2021, 35(4): 523–533. https://doi.org/10.1111/mve.12525
36. Kampango A, Furu P, Sarath DL, Haji KA, Konradsen F, Schiøler KL, et al. Risk factors for occurrence and abundance of Aedes aegypti and Aedes bromeliae at hotel compounds in Zanzibar. Parasit Vectors. 2021;14(1):1–12. https://doi.org/10.1186/s13071-021-05005-9.
37. Saleh F, Kitau J, Konradsen F, Alifrangis M, Lin CH, Juma S, et al. Habitat characteristics for immature stages of Aedes aegypti in Zanzibar city, Tanzania. J Am Mosq Control Assoc. 2018;34(3):190–200. https://doi.org/10.2987/17-6709.1.
38. Rueda LM. Pictorial keys for the identification of mosquitoes (Diptera: Culicidae) associated with Dengue Virus Transmission. Zootaxa. 2004;589(1):1–60. https://doi.org/10.11646/zootaxa.589.1.1.
39. Zymo Research Corporation. DNA/RNA Shield ™. https://zymoresearch.eu/products/dna-rna-shield. Accessed 15 May 2024.
40. Barton B, Peat J, Medical Statistics. A guide to SPSS, Data Analysis and critical Appraisal. Chichester: John Wiley & Sons Ltd.; 2014. (United Kingdom).
41. Kabir A, Zilouchian H, Younas MA, Asghar W. Dengue detection: advances in diagnostic tools from Conventional Technology to Point of Care. Biosensors. 2021;11(7):1–28. https://doi.org/10.3390/bios11070206.
42. Hegde SS, Bhat BR. Biosensors and bioelectronics: dengue detection : advances and challenges in diagnostic technology. Biosens Bioelectronics: X. 2022;10:1–9. https://doi.org/10.1016/j.biosx.2021.100100.
43. Mburu CM, Bukachi SA, Shilabukha K, Tokpa KH, Ezekiel M, Fokou G, et al. Determinants of treatment-seeking behavior during self-reported febrile illness episodes using the socio-ecological model in Kilombero District, Tanzania. BMC Public Health. 2021;21:1–11. https://doi.org/10.1186/s12889-021-11027-w.
44. Mhalu G, Weiss MG, Hella J, Mhimbira F, Mahongo E, Schindler C, et al. Explaining patient delay in healthcare seeking and loss to diagnostic follow-up among patients with presumptive tuberculosis in Tanzania: a mixed-methods study. BMC Health Serv Res. 2019;19:1–14. https://doi.org/10.1186/s12913-019-4030-4.
45. Kassile T, Lokina R, Mujinja P, Mmbando BP. Determinants of delay in care seeking among children under five with fever in Dodoma region, central Tanzania: a cross-sectional study. Malar J. 2014;13:1–10. https://doi.org/10.1186/1475-2875-13-348.
46. Word Health Organisation. Handbook for Clinical Management of Dengue. World Health Organization. 2012. https://www.who.int/publications/i/item/9789241504713. Accessed 15 May 2024.
47. Sekaran SD. Laboratory diagnosis of Dengue: a review. Int Med J Malaysia. 2015;14(1):17–28. https://doi.org/10.31436/imjm.v14i1.452.
48. Elfving K, Shakely D, Andersson M, Baltzell K, Ali AS, Bachelard M, et al. Acute uncomplicated febrile illness in children aged 2–59 months in Zanzibar - Aetiologies, Antibiotic Treatment and Outcome. PLoS ONE. 2016;11:1–21. https://doi.org/10.1371/journal.pone.0146054.
49. Faustine NL, Sabuni EJ, Ndaro AJ, Paul E, Chilongola JO. Chikungunya, Dengue and West Nile Virus Infections in Northern Tanzania. J Adv Med Med Res. 2017;24(4):1–7. https://doi.org/10.9734/JAMMR/2017/37234.
50. Vairo F, Nicastri E, Yussuf SM, Cannas A, Meschi S, Mahmoud MAA, et al. IgG against Dengue Virus in healthy blood donors, Zanzibar, Tanzania. Emerg Infect Dis. 2014;20(3):465–8. https://doi.org/10.3201/eid2003.130150.
51. Ali MA, James OC, Mohammed AA, Joackim A, Mubi M, Omodior O. Etiologic agents of Fever of unknown origin among patients attending Mnazi Mmoja Hospital, Zanzibar. J Community Health. 2020;45:1073–80. https://doi.org/10.1007/s10900-020-00832-w.
52. Mugabe VA, Inlamea OF, Ali S, Maholela P, Melchior B, Muianga AF, et al. Surveillance for arboviruses and leptospirosis among non-malarial acute febrile illness outpatients in areas affected by cyclones Idai and Kenneth in Mozambique. Front Trop Dis. 2023;4:1–7. https://doi.org/10.3389/fitd.2023.1091545.
53. Mulakoli F, Gachara G, Ndombi E, Khamadi S. Prevalence of Dengue virus among healthy blood donors in Mombasa County, Kenya. Pan Afr Med J One Heal. 2024;13(6):1–16. https://doi.org/10.11604/pamj-oh.2024.13.6.42602.
54. Tchuandom SB, Tchadji JC, Tchouangueu TF, Biloa MZ, Atabonkeng EP, Fumba MIM, et al. A cross-sectional study of acute dengue infection in paediatric clinics in Cameroon. BMC Public Health. 2019;19(1):1–7. https://doi.org/10.1186/s12889-019-7252-9.
55. Ngingo BL, Mboera LEG, Chengula A, Machelle I, Makange MR, Msolla M, et al. Aedes aegypti abundance, larval indices and risk for dengue virus transmission in Kinondoni district, Tanzania. Trop Med Health. 2022;50:1–8. https://doi.org/10.1186/s41182-021-00395-z.
56. Musa AA, Muturi MW, Musyoki AM, Ouso DO, Oundo JW, Makhulu EE, et al. Arboviruses and blood meal sources in zoophilic mosquitoes at human-wildlife interfaces in Kenya. Vector-Borne Zoonotic Dis. 2020;20(6):444–53. https://doi.org/10.1089/vbz.2019.2563.
57. Ali EOM, Babalghith AO, Bahathig AOS, Dafalla OM, Al-Maghamsi IW, Mustafa NEAG, et al. Detection of Dengue Virus from Aedes aegypti (Diptera, Culicidae) in field-caught samples from Makkah Al-Mokarramah, Kingdom of Saudi Arabia, using RT-PCR. Front Public Heal. 2022;10:1–7. https://doi.org/10.3389/fpubh.2022.850851.
58. Kaura T, Sarkar S, Sharma V, Mewara A, Devi S, Singh NI, et al. Molecular detection of dengue and chikungunya viruses in surveillance of wild-caught Aedes mosquitoes in Punjab, North India. Trans R Soc Trop Med Hyg. 2024;118(2):95–101. https://doi.org/10.1093/trstmh/trad054.
59. Isa I, Ndams IS, Aminu M, Chechet G, Dotzauer A, Simon AY. Genetic diversity of Dengue virus serotypes circulating among Aedes mosquitoes in selected regions of northeastern Nigeria. One Heal. 2021;13:1–10. https://doi.org/10.1016/j.onehlt.2021.100348.
60. Ward T, Samuel M, Maoz D, Runge-Ranzinger S, Boyce R, Toledo J, et al. Dengue data and surveillance in Tanzania: a systematic literature review. Trop Med Int Heal. 2017;22(8):960–70. https://doi.org/10.1111/tmi.12903.
61. Sintayehu DW, Tassie N, De Boer WF. Present and future climatic suitability for dengue fever in Africa. Infect Ecol Epidemiol. 2020;10(1):1–7. https://doi.org/10.1080/20008686.2020.1782042.
62. Chipwaza B, Mugasa JP, Selemani M, Amuri M, Mosha F, Ngatunga SD, et al. Dengue and Chikungunya Fever among viral diseases in Outpatient Febrile Children in Kilosa District Hospital, Tanzania. PLoS Negl Trop Dis. 2014;8(11):1–11. https://doi.org/10.1371/journal.pntd.0003335.
63. Proesmans S, Katshongo F, Milambu J, Fungula B, Mavoko HM, Ahuka-Mundeke S, et al. Dengue and chikungunya among outpatients with acute undifferentiated fever in Kinshasa, Democratic Republic of Congo: a cross-sectional study. PLoS Negl Trop Dis. 2019;13(9):1–16. https://doi.org/10.1371/journal.pntd.0007047.
64. Mwanyika GO, Sindato C, Rugarabamu S, Rumisha SF, Karimuribo ED, Misinzo G, et al. Seroprevalence and associated risk factors of chikungunya, dengue, and Zika in eight districts in Tanzania. Int J Infect Dis. 2021;111:271–80. https://doi.org/10.1016/j.ijid.2021.08.040.

Publisher's note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.