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Circulation of dengue serotype 1 viruses during the 2019 outbreak in Dar es Salaam, Tanzania

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ABSTRACT

Dengue is an important mosquito-borne viral disease in humans in tropical and subtropical countries. In 2019, a total of 6917 dengue cases were reported in Tanzania based on serological analysis. The aim of this study was to confirm the presence of dengue virus (DENV) and conduct its genetic characterization. A total of 191 serum samples were collected from the outpatients seeking care from health facilities in Kinondoni and Ilala districts between March and May 2019. All the samples were initially tested for the presence of non-structural protein 1 and anti-DENV immunoglobulin G (IgG) and IgM using a commercial OnSite Duo Dengue Ag-IgG/IgM rapid test. Of the 191 sera, 110 (57.6%) were DENV seropositive. The presence of DENV ribonucleic acid was confirmed in 18.2% of the seropositive sera by reverse transcription polymerase chain reaction (RT-PCR). The RT-PCR products were cleaned and partial sequences of DENV polyprotein gene determined using dideoxynucleotide cycle sequencing followed by phylogenetic analysis. We present the occurrence of DENV serotype 1 (DENV-1) during the 2019 outbreak in Tanzania. The DENV-1 strains reported in the present study are highly identical and cluster with Asian DENV-1 strains indicating the possibility of intercontinental spread of DENV through globalization. We advocate for the need for molecular surveillance of dengue viruses during outbreaks to provide rapid evidence of the disease to guide public health interventions.

KEYWORDS

Dengue virus; serotype 1; outbreak; Tanzania

Introduction

In recent years, dengue has become an important mosquito-borne viral disease affecting more than 100 countries in the tropical and subtropical regions of the world, with 50 to 100 million people infected annually [1]. In Africa, the disease is endemic in 34 countries [2], with the most recent outbreaks reported in the East African countries of the Comoros, Ethiopia, Kenya, Mauritius, Seychelles, and Tanzania that share common trade and transport networks [3,4]. In Tanzania, a dengue outbreak was reported for the first time in 1823 in the Islands of Zanzibar [5]. Over the last decade, five dengue outbreaks have occurred in 2010, 2013, 2014, 2018 and 2019 [4,6–8]. From January to October 2019, the total confirmed cases of dengue outbreak from the beginning of outbreak were 6917 cases and 13 deaths translating to 0.2% case fatality rate. The Dar es Salaam region accounted for the majority of cases during this phase of outbreak [4]. Other regions of the country affected included: Arusha Tanga, Dodoma, Kagera, Lindi, Morogoro, Pwani and Ruvuma [4]. Moreover, several studies have reported dengue to be prevalent in several regions of Tanzania including Iringa, Kilimanjaro, Manyara, Morogoro, Pemba and Zanzibar [9–12].

There are four antigenically distinct DENV serotypes; DENV-1, DENV-2, DENV-3, and DENV-4 that share 60–80% homology. Each serotype can cause mild febrile illness to severe forms of the disease known as dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) [13,14]. Since 1960, all four DENV serotypes have been reported in Africa, with DENV-2 epidemics dominating, followed by DENV-1 [2]. In Tanzania, DENV-2 and DENV-3 have been reported during previous outbreaks [6,7,15].

Although dengue is endemic in Africa, genetic characterization of circulating serotypes is not often conducted [16]. The increasing number of dengue outbreaks and improved access to pathogen genome sequencing tools have allowed defining the molecular epidemiology of the disease [17]. Molecular detection is useful for identification of specific pathogen causing epidemics and tracking the sources of outbreaks for appropriate intervention and response. In Tanzania, two studies have reported the evolutionary

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relationships of DENV [7,8]. During the 2013 and 2014 outbreaks, phylogenetic analysis of capsid pre-membrane nucleotide sequences of DENV indicated that DENV-2 was responsible for the outbreaks, that were genetically related to DENV-2 strains reported in China, Indonesia and Singapore [18]. The objective of this study was to confirm the presence of DENV during the 2019 outbreak in Dar es Salaam, Tanzania and conduct its genetic characterization.

Materials and methods

Ethics statement

The study was approved by the Medical Research Coordinating Committee of the National Institute for Medical Research in Tanzania (Ref. No. NIMR/HQ/R.8a/Vol.IX/2974). Written informed consent was obtained from all study participants. Consent was obtained from the parents/guardians for those aged 7–17 years. All the records were documented through tracking forms and handled anonymously.

Study area and design

This cross-sectional health facility-based study was conducted in the Kinondoni and Ilala districts of the Dar es Salaam region in Tanzania. The region usually experiences hot and humid climate throughout the year, with the primary dry season from June to September and short rainy season between October and December followed by long rainy periods between March and May. The average daily temperature is 26°C, and total annual rainfall averages 1110 mm, with a relative humidity of 100% and 60% during the night and daytime, respectively [19]. The study involved four health facilities, namely the International School of Tanganyika, Premier Care Hospital, Doctor's Plaza Hospital, and Regency Medical Center. The facilities are located within Dar es Salaam city serving a diverse population comprising business people, diplomats, and others with high socio-economic interactions. These facilities were purposely selected because they provide routine dengue laboratory testing services. Furthermore, they also provide reference laboratory services for dengue-suspected cases from other health-care facilities within the city.

Sample size and inclusion criteria

The sample size was calculated using a formula described by Arya and Antonisamy [20], assuming 20.9% seroprevalence of DENV infection in Dar es Salaam region [8], and an error rate of 10%. A design effect of three was used to correct for the variability between study districts. The study districts were regarded as clusters and a design effect of three was chosen to obtain an effective sample size of 191 subjects adequate to detect the expected effect. The clinicians recruited patients with dengue-like illness and fever (temperature ≥ 38°C), presenting with at least one of the following clinical signs: retro-orbital pain, rash, arthralgia, malaise, signs of persistent vomiting, severe hemorrhage and organ failure. Febrile patients with bacterial infections and those who were unwilling to participate in the study were excluded. All dengue cases were categorized clinically either as dengue with/without warning signs and severe dengue according to the World Health Organization classification scheme [21].

Sample collection and experimental approach

Serum samples from dengue-suspected outpatients were obtained from selected health facilities from March to May 2019. All the samples were initially tested using OnSite Duo Dengue Ag-IgG/IgM rapid test (CTK BIOTECH Inc, CA, USA), according to the manufacturer's instructions. The sera samples were placed into sterile cryotubes labeled with a unique identification and stored temporarily at −20°C. Thereafter, the samples were transported in dry-ice to the laboratory at Sokoine University of Agriculture in Morogoro, where they were stored at −80°C until analyzed. The sample flow and experimental approach of this study are shown in Figure 1.

Viral RNA extraction

RNA was extracted from 140 µL of serum using a QIAamp RNA mini kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions. The purified RNA was immediately stored in three aliquots each of 10 µL at −20°C until used in order to avoid freeze-thaw cycles that could damage viral RNA. The quality of RNA was determined using a NanoDrop ND1000 spectrophotometer at 260 and 280 absorbance units (GE Healthcare, Buckinghamshire, UK).

Detection and serotyping of DENV by RT-PCR

A conventional one-step reverse transcription polymerase chain reaction (RT-PCR) was conducted on a Mastercyler nexus gradient thermocycler (Eppendorf, Hamburg, Germany) for the detection of DENV using Superscript III Platinum/*Taq* DNA polymerase (Invitrogen, Carlsbad, CA, USA), according to the manufacturer's instructions. The primers, previously described by Lanciotti and others [22], were used to detect the DENV polyprotein gene region that encodes capsid pre-membrane protein (CprM) followed by serotype identification using serotype-specific primers (Table 1). RT-PCR was performed in a 25 µL reaction

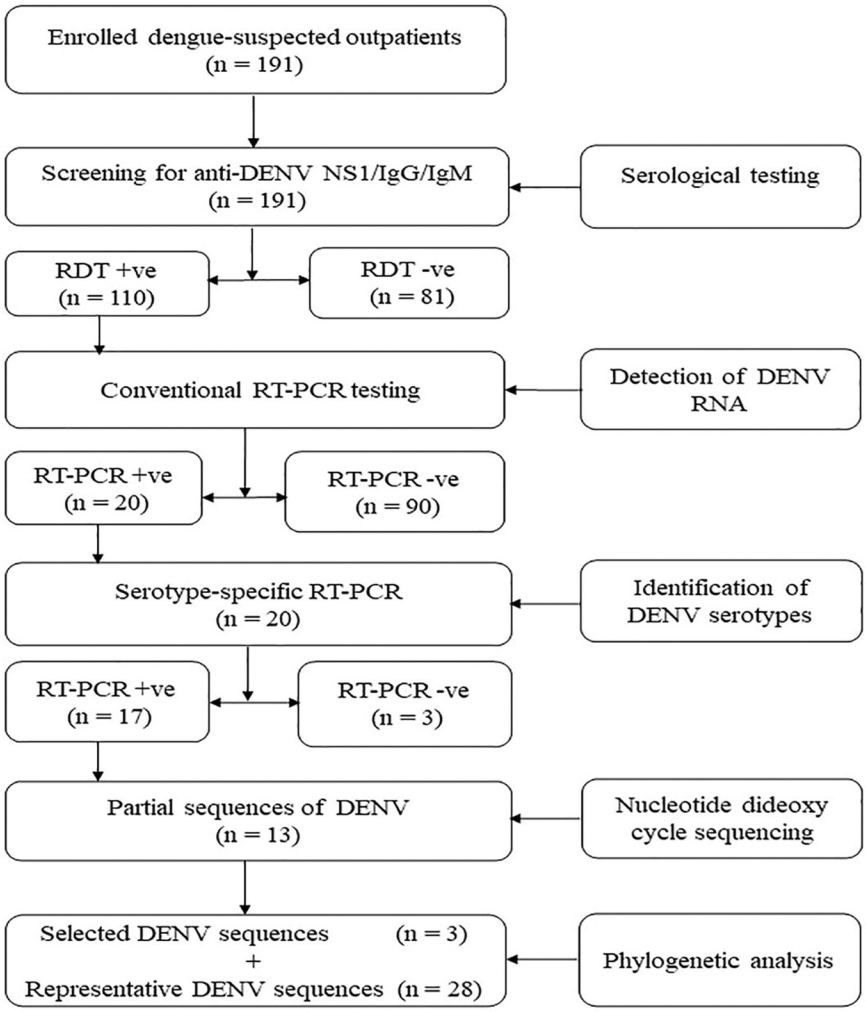

Figure 1. Sample flow and experimental approach used in this study. The chart illustrates the methods for screening non-structural protein 1 (NS1) and anti-DENV immunoglobulin G (IgG) and IgM using a commercial OnSite Duo Dengue Ag-IgG/IgM rapid test (CTK BIOTECH Inc, CA, USA), detection of DENV RNA by conventional reverse transcription polymerase chain reaction (RT-PCR) and genetic characterization after sequencing and phylogenetic analysis.

Table 1. List of primers used in conventional RT-PCR.

Primer name	Sequence ('5–3')	Serotype specificity	Genome position	Amplicon size (bp)	Reference
D1	TCAATATGCTGAAACGCGCGAGAAACCG	DENV-1-4	134–161	511	[22]
D2	TTGCACCAACAGTCAATGTCTTCAGGTTC	DENV-1-4	616–644	511	[22]
TS1	CGTCTCAGTGATCCGGGGG	DENV-1	581–599	482	[22]
TS2	CGCCACAAGGGCCATGAACAG	DENV-2	232–252	119	[22]
TS3	TAACATCATCATGAGACAGAGC	DENV-3	400–421	290	[22]
TS4	CTCTGTTGTCTTAAACAAGAGA	DENV-4	506–527	392	[22]

mix containing 12.5 µL of 2x reaction mix, 1 µL of Superscript III RT/Platinum *Taq* mix, 0.5 µL of 10 µM sense primer (D1), 0.5 µL of 10 µM anti-sense primer (D2), 0.5 µL magnesium sulfate ($MgS0_4$), 4 µL of RNA template and 6 µL of nuclease-free water. Reverse-transcription reaction was performed in one cycle at 48°C for 30 minutes, followed by one cycle of initial denaturation and inactivation of RT-PCR at 94°C for 2 minutes. Amplification was conducted in 35 cycles, each consisting of a denaturation at 94°C for 15 seconds, annealing at 55°C for 30 seconds and elongation at 68°C for 60 seconds. A final extension was performed at 68°C for 5 minutes. Serotype-specific PCR was performed in a 25 µL reaction containing, 12.5 µL of 2x reaction mix, 1 µL of Superscript III RT/Platinum *Taq* mix, 0.5 µL of 10 µM forward primer (D1) and 0.5 µL of 10 µM of each reverse serotype-specific primer TS1, TS2, TS3 and TS4, 0.5 µL magnesium sulfate ($MgS0_4$), 2 µL of initial RT-PCR products (1:5 dilution) and 6.5 µL of nuclease-free water. Amplification was conducted with an initial denaturation at 94°C for 2 minutes followed by 35 cycles, each consisting of denaturation at 94°C for 15 seconds, annealing at 55°C for 30 seconds and elongation at 72°C for 60 seconds

with a final extension at 72°C for 7 minutes. The PCR amplicons were separated on 1.5% agarose gel, stained with Gel Red (Phenix Research Products, Candler, NC, USA), visualized and imaged using a Gel Doc EZ Imager system (Bio-Rad Laboratories Inc, CA, USA).

Sequencing and phylogenetic analysis

The partial genome sequences of DENV in the capsid pre-membrane junction region (CprM, 482 nucleotides) of the polyprotein gene were determined using dideoxynucleotide cycle sequencing using an ABI 3730 Genetic Analyzer (Applied Biosystems, MA, USA). The quality of nucleotide sequences was observed using a sequence scanner software (v2.0) (Applied Biosystems, MA, USA). Consensus nucleotide sequences from the forward and reverse nucleotide sequences were created in BioEdit software (v7.2) [19]. A comparison with homologous sequences available at the National Center for Biotechnology Information (NCBI) database was done using the BLAST nucleotide (BLASTn) online tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Multiple sequence alignments were created using CLUSTAL W tool [24], involving 31 DENV nucleotide sequences selected on the basis of four inclusion criteria; (i) three representative DENV nucleotide sequences (n = 3) selected from 13 identical sequences (99.7% to 100% sequence identity) generated in this study as target sequences, (ii) closest match DENV nucleotide sequences (n = 8) from BLASTn search (99.7% to 100% nucleotide identity), (iii) DENV nucleotide sequences (n = 2) from previous outbreaks in Tanzania to indicate serotype shift, (iv) representative DENV nucleotide sequences (n = 18) from all the four DENV serotypes (DENV1-4) reported from countries in different continents including neighboring countries for global and regional comparison. The phylogenetic relationship was inferred by the Maximum Likelihood method using the General Time Reversible model with a gamma distribution and a fraction of invariant sites (GTR +G5 + I). The reliability of phylogenetic analysis was evaluated with 1000 bootstrap replicates. Furthermore, we computed genetic distances to estimate the evolutionary divergence between pairs of nucleotide sequences. All analyses were performed in Molecular Evolutionary Genetics Analysis software (MEGA v7) [25].

Data analysis

Socio-demographic and serological data were entered into Microsoft Excel spreadsheet (MS-Excel 2016, Microsoft Corp., and Redmond, WA, USA). Descriptive analysis was conducted and results were presented in tables and figures.

Results

Socio-demographic characteristics and serological results

The age of dengue patients ranged from 16 to 75 years (Median = 35 years, IQR = 18). Two-third (67.5%) of the patients were males, 63.9% had post-secondary education and 42.9% had formal employment. Of the 191 sera collected, 57.6% were positive for OnSite Duo Dengue Ag-IgG/IgM rapid test with a larger proportion tested positive for IgM and IgG (17.8%) followed by NS1 and IgM (14.7%) combined tests. High dengue incidence was observed among individuals aged 35 to 44 years, males, those with post-secondary education and those with a formal employment (Table 2).

Table 2. Socio-demographic characteristics of the participants and serological results.

Characteristic	No participants (%)	Seropositive cases*						
Age group (Years)		NS1	IgM	NS1+ IgM	IgG	IgM+IgG	NS1+ IgM+IgG	Total cases (%)
< 18	17 (8.9)	1	2	3	1	2	3	12 (6.3)
18–24	22 (11.5)	0	3	1	1	5	2	12 (6.3)
25–34	38 (19.9)	2	5	6	4	7	2	26 (13.6)
35–44	65 (34.0)	3	7	11	3	11	4	39 (20.4)
45–54	23 (12.0)	2	1	1	0	6	1	11 (5.8)
≥ 55	26 (13.6)	0	1	6	0	3	0	10 (5.2)
Gender								
Male	129 (67.5)	6	16	26	6	21	3	78 (40.8)
Female	62 (32.5)	2	3	2	3	13	9	32 (16.8)
Education								
None	5 (2.6)	0	0	0	3	1	0	4 (2.1)
Primary	5 (2.6)	1	0	0	1	3	0	5 (2.6)
Secondary	59 (30.9)	5	2	11	1	19	0	38 (19.9)
College/University	122 (63.9)	2	17	17	4	11	12	63 (32.9)
Occupation								
Self-employed	57(29.5)	2	8	13	2	9	1	35 (18.3)
Business	16 (8.4)	1	3	2	1	2	1	10 (5.2)
Formal employment	82 (42.9)	4	5	6	4	17	9	45 (23.6)
Students	36 (18.8)	1	3	7	2	6	1	20 (10.5)
Total (%)	191	8 (4.2%)	19 (9.9%)	28 (14.7%)	9 (4.7%)	34 (17.8%)	12 (6.3%)	110 (57.6%)

*NS1, DENV non-structural protein 1 antigen; IgM, anti-DENV immunoglobulin M; IgG, anti-DENV immunoglobulin IgG.

Detection of DENV RNA and serotypes

A total of 20 samples (18.2%, n = 110 seropositive) were positive for DENV RNA by conventional RT-PCR. Of the 20 DENV RNA positive samples, 17 were positive for DENV-1 and three were negative due to low RNA titers after serotype-specific RT-PCR test. The serotype-specific RT-PCR tests for DENV-2, DENV-3 and DENV-4 serotypes were all negative (Table 3).

Phylogenetic analysis of DENV

DENV partial genome sequences of the polyprotein gene obtained in this study were submitted to GenBank and assigned with accession numbers, MT835384, MT835385 and MT835386 (Table 4). Phylogenetic analysis showed that the Tanzanian DENV strains clustered into DENV-1 (Figure 2). The Tanzanian DENV strains had nucleotide identity of 99.7% with Indian DENV strains reported in 2015, 2016, 2017 and 2018, and had 100% nucleotide identity with 2019 Chinese DENV-1 strains (Table 5).

Discussion

Dengue has emerged as a significant public health problem in Tanzania that has evolved from small outbreaks previously seen to large outbreaks experienced in recent years. We performed serological rapid tests to screen for DENV and determined genetic characterization of DENV detected in serum samples obtained from outpatients who presented at health facilities in Kinondoni and Ilala districts of Tanzania.

Our results showed that the age-specific incidence was high among individuals aged 35 to 44 years. This observation indicates low endemicity of the virus in the study area. In highly endemic settings in South-East Asian region, dengue fever occurs mostly in children as adults have acquired immunity following sustained exposure over decades [26]. High vulnerability to DENV infection among this age group may be due to active involvement in socio-economic activities that increase the chances of exposure. These findings are consistent with the reports from other studies [27,28].

A large proportion of sera collected during the outbreak phase was seropositive for anti-DENV IgM and IgG combined tests followed by NS1 antigen and IgM combined tests. These results suggest that most patients presented at health-care facilities during the late acute phase of infection (≥5 days after the onset of fever). During this phase, both NS1 antigen and IgM/IgG antibodies against DENV can be detected in the blood of infected patients [27].

To the best of our knowledge, this study reports DENV-1 for the first time in Tanzania, and thus provides evidence of active DENV transmission and identified serotypes circulating in the country. DENV-1 infections have increased persistently across the globe in the past four decades [29]. In Africa, DENV-1 is among the serotypes that cause frequent epidemics [2]. Phylogenetic analysis results from this study show that viruses from Tanzania had a close evolutionary relationship with the DENV-1 strains from China and India, indicating a possibility for intercontinental spread. In comparison, a recent study in Japan has also reported DENV-1 strain isolated from the patient who had traveled to Tanzania during May 2019 [30].

Table 3. The characteristics of RT-PCR positive sera samples.

Sample	District	Location*	Sex	Age	RNA quality	DENV RNA test	DENV-1 test	DENV-2 test	DENV-3 test	DENV-4 test
P1	Kinondoni	RMC	M	16	2.83	+[a]	DENV-1	–[b]	–[b]	–[b]
P2	Kinondoni	RMC	M	48	2.25	+	DENV-1	–	–	–
P3	Ilala	DPH	M	45	1.54	+	DENV-1	–	–	–
P4	Kinondoni	IST	F	34	2.75	+	DENV-1	–	–	–
P5	Kinondoni	DPH	F	36	1.42	+	–[b]	–	–	–
P6	Ilala	RMC	M	56	2.09	+	DENV-1	–	–	–
P7	Ilala	RMC	M	26	2.45	+	DENV-1	–	–	–
P8	Kinondoni	DPH	M	35	2.22	+	DENV-1	–	–	–
P9	Kinondoni	DPH	M	44	1.57	+	DENV-1	–	–	–
P10	Ilala	PCH	M	40	1.66	+	DENV-1	–	–	–
P11	Ilala	PCH	M	65	4.98	+	DENV-1	–	–	–
P12	Kinondoni	IST	M	29	5.39	+	DENV-1	–	–	–
P13	Ilala	RMC	M	22	1.69	+	–	–	–	–
P14	Kinondoni	DPH	M	33	2.81	+	DENV-1	–	–	–
P15	Kinondoni	DPH	M	20	2.61	+	DENV-1	–	–	–
P16	Kinondoni	PCH	M	27	1.53	+	–	–	–	–
P17	Kinondoni	PCH	M	21	1.95	+	DENV-1	–	–	–
P18	Ilala	IST	F	37	2.00	+	DENV-1	–	–	–
P19	Kinondoni	RMC	M	21	2.14	+	DENV-1	–	–	–
P20	Kinondoni	RMC	F	31	2.43	+	DENV-1	–	–	–

*RMC, Regency Medical Center; DPH, Doctor's Plaza Hospital; IST, International School of Tanganyika Clinic; PCH, Premier Care Hospital; [a] RT-PCR positive for universal DENV RNA test; [b] Serotype-specific RT-PCR negative test; DENV-1, dengue virus serotype 1; DENV-2, dengue virus serotype 2; DENV-3, dengue virus serotype 3; DENV-4, dengue virus serotype 4.

Table 4. Representative DENV virus isolates/strains used for reconstruction of phylogenetic tree.

s/n	DENV isolate/strain	Country	Year	Serotype	Genetic distance*	Accession no	Reference
1	DENV-1/TAN/DAR-S1/2019	Tanzania	2019	DENV-1	0.000	MT835384	This study
2	DENV-1/TAN/DAR-S2/2019	Tanzania	2019	DENV-1	0.000	MT835385	This study
3	DENV-1/TAN/DAR-S3/2019	Tanzania	2019	DENV-1	0.004	MT835386	This study
4	19XN09422	China	2019	DENV-1	0.000	MN923102.1	NCBI**
5	19XN08100	China	2019	DENV-1	0.000	MN923101.1	NCBI
6	19XN51128	China	2019	DENV-1	0.000	MN923096.1	NCBI
7	19XN25802	China	2019	DENV-1	0.000	MN923085.1	NCBI
8	NU1876	India	2018	DENV-1	0.004	MK796420.1	NCBI
9	STM1818	India	2017	DENV-1	0.004	MT126440.1	NCBI
10	STM1369	India	2016	DENV-1	0.004	MT126438.1	NCBI
11	STM20778	India	2015	DENV-1	0.004	MT126436.1	NCBI
12	01/21,123	Brazil	2011	DENV-1	0.042	JN092559.1	NCBI
13	Comoros 04.329/93	Comoros	1993	DENV-1	0.030	DQ285562.1	NCBI
14	Angola_2013	Angola	2013	DENV-1	0.090	KF184975	[33]
15	295arg00	Argentina	2002	DENV-1	0.038	AF514885.3	[34]
16	19,492	Singapore	2016	DENV-1	0.004	MF033253.	[35]
17	2006Den-1	India	2006	DENV-1	0.042	EF080814.2	[36]
18	MG-2/2013	Brazil	2013	DENV-1	0.054	KM093798.1	NCBI
19	22,125	Singapore	2015	DENV-1	0.007	MF033237.1	[35]
20	C	Brazil	2010	DENV-2	0.574	JN086992.1	[37]
21	DENV2/TAN/Muhimbili/2014	**Tanzania**	**2014**	DENV-2	0.550	KM892496.1	[18]
22	DENV2/TAN/IFM/2013	**Tanzania**	**2014**	DENV-2	0.550	KM892493.1	[18]
23	MDU140	India	2017	DENV-2	0.697	LR595983.1	NCBI
24	21/RMRC/Orissa/2011	India	2011	DENV-3	0.412	JQ717295.1	[38]
25	BH-1/2006	Brazil	2006	DENV-3	0.404	GQ330909.1	[39]
26	Strain 251,991	Kenya	1991	DENV-3	0.404	AF547239.1	[40]
27	89-SriLan3	Sri Lanka	2016	DENV-3	0.378	AF547232.1	[40]
28	IND/JIPMER/2866	India	2017	DENV-4	0.697	MH431924.1	NCBI
29	PHR3C10D4/2014	Philippines	2014	DENV-4	0.764	KT070878.1	NCBI
30	B1008	French Guiana	2006	DENV-4	0.735	EU518596.1	[41]
31	Den-M18/2010	India	2010	DENV-4	0.697	KM507024.1	NCBI

*The number of base substitutions per site from between sequences was estimated using Kimura-2 parameter model [42], **NCBI, National Center for Biotechnology Information nucleotide database available at https://www.ncbi.nlm.nih.gov/nuccore/

Subsequent phylogenetic analysis indicated that the Japanese DENV-1 strain (Accession no LC485151) was genetically related to 22,125 Singapore 2015 strain (Accession no MF033237.1). These findings suggest that DENV-1 strains that caused an epidemic during the 2019 in Tanzania are co-circulating in Asia.

Tanzania interacts with many countries for trading, social and economic activities, thus putting it at a risk of introducing DENV from one country to another. Extensive international travel, unplanned urbanization, infrastructure connectivity and global trade networks are significant factors in facilitating the spread of DENV infection between the affected and non-affected areas [29,31]. The recent spread of DENV-1 cases in the East African region is a growing public health threat. In 2019, WHO reported sporadic DENV-1 cases in the Comoros, Mauritius and Seychelles with evidence of importation from other countries [4]. Since circulation of DENV-2 and DENV-3 serotypes has been previously reported in Tanzania [6,7], occurrence of DENV-1 during the 2019 outbreak underscores the need for continuous molecular surveillance of dengue viruses in Tanzania. In endemic countries, the presence of multiple dengue viruses is associated with an increased risk of severe dengue infections [32]. These observations advocate the importance of global, regional and local surveillance of dengue viruses to inform public health interventions.

It is important to note that under the health facility-based study settings, the subjects may not represent the entire infected population, suggesting that the results should be generalized with caution. As dengue becomes endemic in Tanzania, healthcare providers are increasingly becoming aware of the need to detect the infection and provide prompt appropriate clinical care to patients. It is important that rapid diagnostic kits are made available at both public and private health facilities to ensure people readily get the required services on time.

In conclusion, confirmatory laboratory diagnosis using RT-PCR facilitated the detection of DENV-1 circulation in Tanzania during the 2019 outbreak. Circulation of new or multiple serotypes is likely to lead to increased risk of severe dengue. There is, therefore, a need to establish a continuous dengue surveillance program to detect outbreaks, monitor the spread of serotypes, and determine dengue burden in the country, in space and time. Future complete genome sequencing of circulating dengue viruses in Tanzania will provide a better understanding of the molecular epidemiology of dengue infection and guide strategies for interventions.

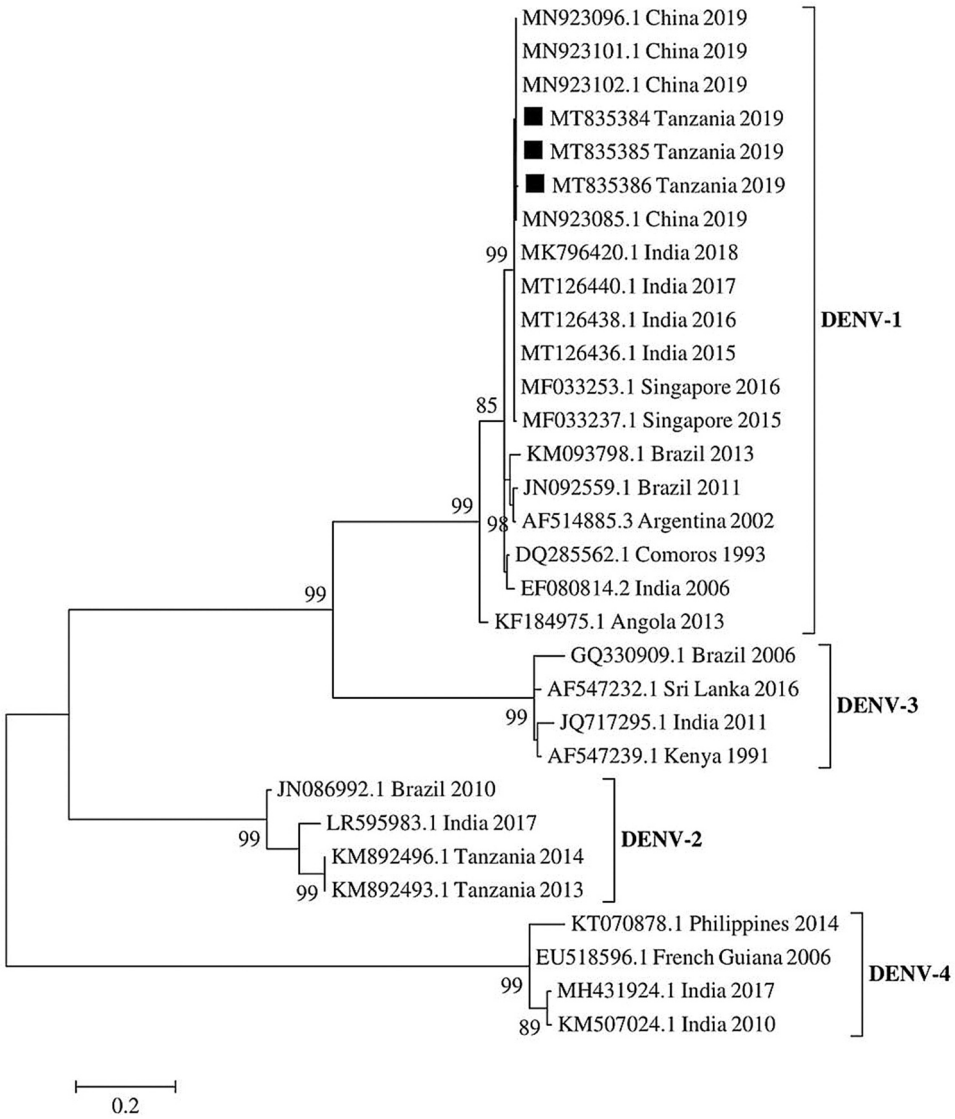

Figure 2. Phylogenetic tree of DENV partial polyprotein gene sequences at capsid pre-membrane junction region (CprM). DENV-1 strains detected during the 2019 outbreak in Tanzania are indicated in black squares. The evolutionary relationship was inferred by the Maximum likelihood method in 1000 bootstrap replicates. The bootstrap support values >80% are shown at the nodes. The scale bar indicates nucleotide substitutions per site.

Table 5. Basic local alignment search results for homologous DENV-1 sequences available at GenBank database.

s/n	Isolate	Country	Accession no	Collection date	Serotype	Nucleotide identity	Reference
1	19XN09422	China	MN923102.1	May 2019	DENV-1	100%	NCBI*
2	19XN08100	China	MN923101.1	Apr 2019	DENV-1	100%	NCBI
3	19XN51128	China	MN923096.1	Oct, 2019	DENV-1	100%	NCBI
4	19XN25802	China	MN923085.1	Oct 2019	DENV-1	100%	NCBI
5	NU1876	India	MK796420.1	Jun 2018	DENV-1	99.79%	NCBI
6	STM1818	India	MT126440.1	Oct 2017	DENV-1	99.79%	NCBI
7	STM1369	India	MT126438.1	Oct 2016	DENV-1	99.79%	NCBI
8	STM20778	India	MT126436.1	Nov 2015	DENV-1	99.79%	NCBI

*NCBI, National Center for Biotechnology Information nucleotide database available at https://www.ncbi.nlm.nih.gov/nuccore/

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Disclosure statements

The authors declare the absence of any competing interests.

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